

The
AMERICAN JOURNAL
of
MEDICAL TECHNOLOGY

VOLUME 15

JULY, 1949

NUMBER 4

THE PREPARATION AND USE OF Rh HAPten

By BETTY BROCKLAND, M.S.,
Firmen Desloge Hospital, St. Louis, Mo.

Even though the Rh factor was established as the etiological agent of erythroblastosis in 1941, still therapy leaves much to be desired and prophylactic measures are not practical. The pediatricians and obstetricians are still at a loss for the treatment of this disease. Lack of specific therapy led to the development of the various forms of transfusions for handling the afflicted infants. Some physicians favor small repeated blood transfusions, others perform massive replacement transfusions. Some insist only Rh-negative blood may be used, others advocate the use of Rh-positive blood. The results are equivocal and there remains the large percentage of stillborn infants and macerated fetuses which are beyond any transfusions or supportive therapy.

Bettina Carter, Immunologist at Western Penn. Hospital, Institute of Pathology, Pittsburgh, Pa., after two years of research has recently reported the isolation of an Rh hapten. According to Landsteiner's definition, a hapten is a specific protein-free substance which, although active in vitro, induced no or only slightly antibody response. Thus a hapten performs as an antigen in that it combines with an antibody in vivo and in vitro, but unlike an antigen it, of itself, will not elicit the production of antibodies.

Carter's method of preparation uses chemical means resulting in a protein-free Rh hapten. The product completely inhibits high titered human anti-Rh serum.

* 1st Award Paper, A.S.M.T. Convention, June, 1949, Roanoke, Va.

Method of Preparation

Several samples of Rh positive red blood cells are pooled to a volume of 1,000 cc. Cells of any or all of the four major blood groups may be used. After the plasma has been drawn from the blood bank the cells are excellent for Rh hapten production. Intact cells are the only requisite, the age of the blood is not the important consideration. Laking of the cells is accomplished by adding half the volume of distilled water, i.e. 500 cc. to 1,000 cc. of red blood cells. Next to precipitate the protein add 4,000 cc. of 95% ethyl alcohol. Mix well and allow this to stand overnight at 4° C. A heavy red precipitate is the result. Filter through a Buchner funnel and discard the filtrate. Wash the cells with 50% ethyl alcohol, filter as before and wash with 25% alcohol. Again filter and discard the filtrate. Extractions with decreasing concentrations of ethyl alcohol remove both the water soluble and the alcohol soluble group specific substances. Then the precipitate is suspended in five volumes of ethyl ether for two to five days. During the entire period the extractions are accomplished at 4° C and mixed each day for a thirty minute period. After five days the precipitate is filtered and discarded. The filtrate containing the hapten dissolved in ether is retained. Evaporation of the ether is accomplished at room temperature with the aid of an electric fan. When the ether has been completely removed by evaporation, the residue is a gummy material, light yellow to dark amber in color. It contains the hapten and is soluble in warm alcohol, ether, chloroform and similar fat solvents. For twenty-four hours the material is subjected to ultraviolet irradiation to eliminate all possible virus contamination. It is dissolved in warm 95% alcohol and sealed in ampules so that each cc. of alcohol contains 100 mg. of hapten.

The Rh hapten is apparently a lipid substance. It is insoluble in water and soluble in warm 95% alcohol, in ether, chloroform and acetone. Price⁵ and his associates at Notre Dame University have proceeded toward the further identification of the chemical nature of Rh hapten. According to a preliminary report in the Journal of the American Chemical Society, the crude extract prepared by Carter's method was freed of phospholipids by precipitation with acetone. Phospholipids comprise 25 to 50% of the crude material. Various fractions containing crude cholesterol were obtained followed by fractions which yielded glistening needles by recrystallization from ether-pentane or chloroform-pentane solution. From 979mgs. of crude extract, 80mg. of pure hapten was obtained.

Pure Rh hapten is described as an acid; optically inactive, soluble in alkali with a melting point of 156.9° to 157.2° with activity in dilutions of 1:5000 as measured by the complement fixation test with anti-rh_o serum.

Another different hapten was contained in the crude cholesterol fraction but it has not been isolated in pure form. It is neutral in reaction and active in dilutions as high as 1:100,000. These two substances may be responsible for two of the Rh subgroups.

To assay hapten² to determine its potency the best method is complement fixation. The test is modeled after Kolmer's sero-diagnostic test for syphilis. A 1% solution of Rh hapten in 95% alcohol is diluted with four volumes of .85% saline to produce a

1:500 dilution. Similar dilutions are made to provide concentrations of 1:1000, 1:1500 and 1:2000. The suspension of hapten in saline gives a milky, opalescent liquid.

For each of these concentrations (1:500, 1:1000, 1:1500, 1:2000) two 13 x 100 mm. tubes are set up in serologic racks, as well as two tubes for a normal serum control for each antigen dilution. A control tube is set up for each of the following: antigen hapten, unsensitized serum, hemolysin and sheep red cells. In the four pairs of tubes for testing hapten, place 0.2 cc. anti-Rh° (anti-D) serum diluted so that its titration end point is 1:32. In the four pairs of tubes set up for control purposes, put 0.2 cc. normal (unsensitized) serum. To each of the eight tubes for the test, add 0.5 cc. of the appropriate dilution of hapten. This means that tubes 1 and 5 will contain 0.5 cc. hapten dilution 1:500, tubes 2 and 6 will carry 0.5 cc. hapten dilution 1:1000 tubes 3 and 7 will have 0.5 cc. of the 1:1500 dilution, and tubes 4 and 8 contain 0.5 cc. of a 1:2000 dilution. To the serum control tube, add 0.5 cc. saline. The antigen controls (one for each hapten dilution) consist of 0.5 cc. saline, plus 0.5 cc. of the appropriate dilution of hapten. The hemolysin control tube receives 1 cc. saline. The sheep red cell control tube contains 2.5 cc. saline.

The tubes are allowed to stand for fifteen minutes when 1 cc. of saline containing two full units of complement (units are determined by previous titration of lyophilized complement according to Kolmer's method) is added to each tube in each series and to each control tube except the sheep red cell control. The racks are shaken thoroughly, but briefly, and are allowed to stand at 4° C. for four hours. At the end of this period, the racks are removed from the refrigerator, placed in a 37°C. waterbath for ten minutes, then to each tube except the red cell control is added 0.5 cc. saline which contains two units anti-sheep cell hemolysin. The hemolysin units have been determined by previous titration. Immediately 0.5 cc. of a two per cent suspension of washed sheep red cells are added to each tube. The tests are put back into the 37°C. waterbath until the controls are completely clear, when readings are made.

To achieve a relative uniformity in relation to the work of several investigators, an arbitrary standard for measuring the activity of Rh hapten was established. Such a standard or a unit may be defined as the least quantity of Rh hapten required to fix two full units of complement in the presence of an anti-D serum possessing an antibody titer of 32 units under the conditions of the test. The titer of 32 units for the serum is chosen since that is the lowest titer indicated by the Nat. Institute of Health as acceptable for Rh testing, e.g. if the smallest amount of Rh hapten of a given lot required to fix complement in the pres-

ence of an anti-Rh serum of 32 units is 250 gamma per cc. then an ampoule containing 250 mg. of hapten per cc. of alcohol may be said to have 1000 units of Rh hapten.

Several cases may be cited to demonstrate the uses of Rh hapten.

Case 1. Mrs. R. Z., 29 years old, white, has been married 6 years. Her first pregnancy in 1944 resulted in a normal male infant after an uneventful antenatal course and spontaneous uncomplicated delivery. Her second pregnancy in 1946 was also entirely without incident and after a short labor and spontaneous delivery a second male child was born but this infant had severe *icterus gravis* and anemia. Unfortunately, Rh antibody studies were not done during this pregnancy. The affected infant was treated with multiple (six) small transfusions and supportive therapy and after three weeks, was discharged as cured. She again became pregnant in September, 1947 but was not seen until the third month of her pregnancy. Rh antibodies were first found in the maternal serum in January, 1948 at which time "blocking antibodies" in a titer of 1:28 were discovered. One month later, the titer had increased to 1:64 and remained at this level during March. At that time Rh hapten was made available to treat this patient and after an initial lag period of one week, the titer fell progressively with treatment until on May 5, 1948, no Rh antibodies could be found. Treatment was continued at weekly intervals. Her expected date of confinement was June 17, 1948. However, on June 2nd, blocking antibodies were again found in the maternal serum in a titer of 1:8. A large dose of Rh hapten (300 mg.) was given and three days later, labor was induced. Blood taken on the morning of induction contained blocking antibodies in a titer of 1:2. Induction resulted in a three hour labor and an 8 pound, 4 ounce male infant was then spontaneously delivered. The *infant was normal* in every respect. No edema or jaundice were present. The placenta was grossly normal and weighed 600 grams. The amniotic fluid was clear and colorless. Immediate blood study disclosed that the child was Rh-positive (Group O; Rh, heterozygous). The red cell count was 6.04 million and the hemoglobin 16 grams. Only one nucleated red cell per 100 white blood cells could be found. Serum from cord blood contained no free Rh antibodies but the erythrocytes gave a weakly positive Coombs test. The child was observed very closely during the next ten days but at no time during this period did the hemoglobin fall below 16 grams nor the red blood cell count below 6 million. On discharge from the hospital on the 6th day of life, the hemoglobin was 16.2 grams and the red blood cells 6.03 million. Since that time, the child has been seen frequently and at three months of age showed no abnormalities. He has maintained his hemoglobin and red blood cell count and has gained adequately.

Case 2. Mrs. C. G., 36 years old, has been married 18 years. In 1933 and again in 1935 she delivered normal babies at term. In 1938 she had pneumonia and was given two blood transfusions, one from her husband, the second from his brother. In 1939 she gave birth to a term child that died on the third day of erythroblastosis fetalis. In 1941 a term child was born that was severely involved but survived with daily, small transfusions of Rh-positive blood from the father. In 1945 a term child was born but it died of erythroblastosis on the second day. In 1946, the patient mis-

carried a 2½ month fetus. No antibody titers could be obtained and it was doubtful whether any studies had been made along these lines. The patient came to us beginning her seventh month of pregnancy and a blood study was made.

Blood	Group	Rh Type	Remarks
Mother	O	Rh Neg.	
Father	O	Rh ₁	Homozygous

At that time her antibody titer was 1:16. Two weeks later her antibody titer was 1:64 and hapten administration was begun. The dosage was usually 200 mg. and during the first four weeks the injections were given at weekly intervals. In an attempt to negate the antibody titer semi-weekly injections were administered till the end of term. After the first injection the antibody titer fell from 1:64 to 1:8, then it rose to 1:16. During the eighth month the titer fluctuated between 16 and 32 and in the final month the titers ranged from 8 to 16. At the time of delivery the titer was 1:16 with no demonstrable blocking antibodies.

The birth was spontaneous, the offspring delivered was a living male weighing about 2500 gms. The red cell count at birth was 4.5 million per cubic millimeter with 35 nucleated red cells per 100 white blood cells. The infant appeared slightly icteric; the icterus index on the cord blood was 14.5 units. An antibody determination on the cord blood revealed a titer of 1:2. An injection of 200 mg. of hapten was administered to the infant in the buttocks. Late on the first day a transfusion of 40 cc. of the father's blood was given after which the red count rose to 5.1 million per cubic millimeter. The hemoglobin was 15 grams. The nucleated red cells were 7 per 100. On the second day the red cell count was 4.7 million per cu. mm. with 5 gm. of hemoglobin. On the fourth day the baby was again transfused with 60 cc. of the father's blood. At this time the baby began to show signs of neurological involvement. A smear from the peripheral blood showed 20 nucleated red cells per 100 white blood cells. Early on the fifth day the baby expired.

The diagnosis of erythroblastosis fetalis was confirmed by autopsy.*

Difference between success and failure may be the time at which treatment is begun. It is believed that the Rh antibodies traverse the placenta after the seventh month of gestation. Therefore, the antibodies must be rendered ineffectual in the mother before that time. It seems that the mode of action of Rh hapten is a neutralization or inactivation of the antibodies themselves since the hapten apparently will not effect a change in fetal damage which has occurred prior to its administration.

The mechanism of the action of Rh hapten can only be guessed. Whether it neutralizes antibody or whether it satisfies antibody receptors only further investigation will determine. In active sensitization, the maternal titer will fall in response to treatment with Rh hapten.

* Case 1 was a patient treated at the University of Minnesota Hospitals. Case 2 was a patient at St. Mary's Hospital, St. Louis, Missouri.

REFERENCES

1. Goldsmith, Joseph W., "Bulletin of the University of Minnesota Hospital," Vol. XX, No. 9, Dec., 1948.
2. Loughery, J. and Carter, B. B., Am. J. Obst. & Gyn., 55: 1948.
3. Carter, B. B., "Studies on the Rh Hapten," Pennsylvania Medical Journal, Vol. 52, p. 124-127, 1948.
4. Carter, B. B., "Rh Hapten: Its Preparation, Assay and Nature," Journal of Immunology, 61, Jan. 1949.
5. Price, Charles C., "The Isolation of a Substance with Rh Hapten Activity," Journ. of Am. Chem. Society, 70, 1948, p. 3527.

THE USE OF ORDINARY TOADS AND FROGS
FOR PREGNANCY TESTS*

LEONIDE B. SOUCY, M.T. (ASCP)

Plainview Sanitarium and Clinic, Plainview, Texas

In March, 1947, a completely new test for pregnancy was described by Galli Mainini¹ of Argentina. The animals used were male toads and, unlike most other biologic pregnancy tests, it required no surgical intervention for observation of results. Readings were made by simple microscopic examinations of the toad's urine, which was obtained directly from the cloaca. The presence or absence of spermatozoa in this urine established the positivity or negativity of the reaction. The author reported that he had found the method to be specific, accurate, rapid, simple and economical.

Later, this same worker published several other papers^{2, 3, 4} on the test. The last of these appeared in an American publication of very wide distribution. It was undoubtedly noted by many laboratory workers, but it evidently did not make a great impression, because the test does not seem to be in wide use.

Perhaps the reason for this lack of popularity lies in the fact that the animals described by the originator of the technique were for the most part species of Salientia not found in this country. This probably led many to believe that the test would not be practical, because it would presumably entail the trouble and expense of importing exotic animals. In February, 1948, however, Wiltberger and Miller⁵ reported that they had applied Galli Mainini's technique to the common Leopard Frog of the United States and found it satisfactory. Robbins and Parker,⁶ in April, 1948, also reported that this North American frog was suitable. During the past year, other groups^{7, 8, 9} have published their observations and experiences with the test. Among other

* 2nd Award Paper, A.S.M.T. Convention, June, 1949, Roanoke, Va.

things, it seems to show great promise as a diagnostic aid in differentiating between threatened and inevitable abortion, or incomplete and complete abortion.^{8, 9}

All of the American reports to date have emphasized, as Galli Mainini did when reporting on toads, that this test was very accurate, rapid, simple and economical. However, many of the details of technique and problems coincident to the adoption of this method were lacking. Since these are the factors with which technologists are mainly concerned and for which they are usually held responsible, it is the purpose of this paper to elaborate on problems and technical details, and to add further to the accumulating evidence regarding the reliability of this new test for the laboratory diagnosis of pregnancy.

The work reported on here consists of observations made during the six-month period, September 14, 1948 to March 10, 1949, and includes the results of 341 tests.

Materials

FROGS—SPECIES: The frogs used in this investigation were of the same species as those used by the American workers previously mentioned; namely, *Rana pipiens*. *Rana pipiens* is most commonly known as the Leopard Frog, Grass Frog or Meadow Frog. Other species, because of their close resemblance to the Leopard Frog, are often included in the *pipiens* group and may be known by the same common names. In addition to the frog, toads were investigated. A few specimens of a native species, *Bufo cognatus*, or Great Plains Toad, were tested, and were found to be the equal of the frog in most respects. It seems likely that almost any species of American toad or frog, providing there is no bizarre life-history, would be suitable for this test.

DISTRIBUTION: *Rana pipiens* is widely distributed throughout the North American continent. Some of the several sub-species may be found in all of the states in this country, and in parts of Canada and Mexico.^{10, 11}

SEX: Only males may be used for the test, and it should go without saying that utmost care should be exercised in selecting animals of the proper sex. The most easily differentiated secondary sex characteristics are found in the thumbs. In the male, the thumb is large—much larger than the other digits—rounded at the base and heavily pigmented; whereas in the female, the thumb is of about the same size as the other digits, tapering and lightly pigmented. At certain times of year the sex characteristics of the male may more closely resemble those of the female, and thus make differentiation more difficult. When there is

doubt, sex may be definitely established by injecting known pregnancy urine.

SIZE: The size of mature males may vary in different geographic locations. This, however, does not seem to be of as great importance as previously reported. The frogs used in this series ranged in weight from 15 to 75 Grams, and the smaller specimens reacted as well and to as many injections as the larger animals.

PROCUREMENT: Most of the frogs were collected locally. For most laboratories, however, the practical method of procurement would be to purchase the frogs from dealers or biological supply houses. Supply catalogs list frogs at 15 to 40 cents each, and advertisements for the sale of these animals now appear in a few medical journals.

MAINTENANCE TANKS—Almost any type of container may be used as a storage tank. Ordinary galvanized home-laundry tubs (Fig. 1) were quite satisfactory. A set of twin-tanks mounted on a single frame, as shown in figure 1, may be purchased at any hardware store for \$10.00 to \$15.00. Those shown here measured 20 x 20 x 11 inches, and could have easily accommodated 200 frogs. Wire screen covers with a small wooden door in the center were constructed to keep the frogs from escaping. The small wooden door was particularly useful in enabling the passage of one arm in the tank without providing an avenue of escape for the animals.

Some sort of water pool seems to be a requisite for the well being of the frogs. To meet this need, water was allowed to drip in emesis basins which were placed in the bottom of the tanks (Fig. 2).

ISOLATION CONTAINERS—The simplest means of observing the frogs after injections is to place them in individual containers. Glass jars or tin cans (Fig. 3) may be used for this purpose. One-gallon fruit and vegetable tin cans, which were obtained from the hospital kitchen after meals, proved quite satisfactory. In many respects, other than economy, tin cans are superior to glass jars for isolation and observation purposes.

SYRINGES AND NEEDLES—These are standard laboratory equipment. Two, five or ten cc syringes may be used for injections, and 24 or 25 gauge needles, 1½ inches in length, are recommended. Larger needles may permit the injected urine to escape at the puncture site.

CATHETERS—Small pieces of scrap glass tubing, 3 mm to 7 mm in diameter, may be drawn to a capillary end and utilized as catheters for collecting urine from the frogs (Fig. 6).

The only other items of equipment necessary for the test are glass slides and a microscope.



FIG. 1



FIG. 4



FIG. 2



FIG. 5



FIG. 3



FIG. 6

Maintenance

STORAGE SPACE: Since frogs have not been incriminated as vectors of human disease, impart no odors, make little or no noise in captivity, and devour insects which may venture into the cages, their presence in or near the laboratory—or hospital or public buildings—should not be objectionable.

The choice of an animal room then presents no special problem. It may be indoors or outdoors; in the laboratory, in the basement, or on the roof.

TEMPERATURE: If an indoor room is selected, the temperature should not exceed 100° F.; if outdoors, the temperature must always be above freezing. No difference in end-results and no appreciable differences in reaction times were noted between

large groups of frogs kept at 40 to 60° F. for several weeks and groups kept at 90 to 100° F. for the same period of time. Low temperatures (40 to 60° F.) are usually recommended because the frogs are less susceptible to disease in cold atmospheres.

DISEASE: Probably the only disease frequently fatal to frogs and other amphibians is "red-leg." Epidemic outbreaks of "red-leg" often occur among frogs kept in captivity, and may easily decimate a colony in a few days. It might be noted that none of the previous reports on this test has mentioned this troublesome disease. The technologist who acquires a colony of frogs without a knowledge of "red-leg" will most likely suffer many disappointments. The scope of this paper permits only a brief discussion of the disease.

A bacillus, *Proteus hydrophilus fuscus*, is usually accused as the etiological agent.

The chief characteristics of the disease are sluggishness of the animals, dullness of the skin and excessive secretions of mucus, inability of the frogs to raise their heads, and in the terminal stages, petechial hemorrhages or redness of the legs and ventral surfaces, and finally bloody vomitus.

Frogs showing any of these symptoms should be discarded, or placed in a refrigerator at temperatures just above freezing for a week or more. The unaffected animals should be washed in running water, and the tanks should be scrubbed with a good disinfectant. If practicable, a good means of preventing "red-leg," is to pack all newly purchased frogs in ice for about a week after their arrival. They should then be washed in fast flowing water for three or four days.

The treatment and prevention of this disease is described in an excellent paper, by Emerson and Norris,¹² which was written almost half a century ago. The account is quite detailed and is highly recommended for all those working with amphibians. By following the methods of these two authors, the one epidemic of "red-leg" occurring during this investigation was completely controlled in a few days and recurrences have been prevented even though the frogs are now kept at 65 to 85° F.

Most frogs are hosts to enormous numbers of helminths and protozoa. In the course of examinations for spermatozoa, various parasitic forms may be found. These parasites, however, do not seem to have serious detrimental effects on the frogs, nor are they found to be pathogenic for man.

LIGHT: Light does not seem to be a factor. One group of 30 frogs was exposed to constant artificial light for eight days, and another group of 30 was kept in total darkness for the same period of time. The reaction times were about the same for both groups.

DIET: Frogs and toads may endure fasting periods of several weeks, or even several months. To be used repeatedly for pregnancy tests, however, they should be fed at least once a week. If deprived of food, they may become less resistant to disease and may not react properly.

They will reach for almost any small object, providing it is in motion. They will not touch dead or motionless insects. If dead insects or pupae are available, they may be set in motion by flicking them with a glass rod or broomstraw, but this practice may result in injury to the frogs.

The frogs may also be force-fed,¹³ but since this is a time consuming process it is not recommended.

The frogs used in this series were maintained on a diet of "mealworms" (the larvae of *Tenebrio* beetles). Mealworms may easily be reared in the laboratory, or they may be purchased from most biological supply houses. They are clean, odorless, easy to handle, and readily accepted by the frogs; though it may take the frogs a few days to learn to catch the worm.

The feeding costs amount to less than a penny for each frog per week.

It is best to feed the frogs only on week-ends, or only those frogs which will not be used for a test for 3 or 4 days. They will not tolerate injections on a full stomach. They regurgitate after injections, and may choke in the process if the stomach is full.

Technique

The entire procedure includes only five simple steps: (1) Untreated urine from the woman suspected to be pregnant is injected into the dorsal lymph sac of an adult male frog; (2) The animal is placed in a properly labeled jar or can containing sufficient water to cover the bottom; (3) Thirty minutes to two hours later, urine is obtained from the frog by introducing a capillary pipette into the cloaca; (4) One drop of this urine is placed on a clean glass slide without coverslip; (5) The drop of urine is examined under the low power of the microscope for spermatozoa. If spermatozoa are found, the test is positive. If no sperms are seen within two hours after injections, the test is negative.

TYPES OF URINE SPECIMENS: No special preparation of the patient or urine is required, except that a first morning specimen should probably be used. In most cases, urine collected at any time of day will give reliable results. Several positive reactions have been observed in thirty minutes with urine of very low specific gravity, but the one false negative test reported was obtained with urine collected in the afternoon. It seems advisable to always check negative mid-day urine with a first

morning specimen, though further investigations may prove that this is not necessary.

Voided specimens are quite satisfactory and there seems to be no reason to subject the patient to the discomfort of catheterization.

TOXICITY: Frogs have been injected with urine containing large quantities of pus, blood, mucus, casts, bacteria, fungi, trichomonads, menstrual discharges, human spermatozoa, normal and abnormal crystalline elements, albumin and sugar, without affecting the animals or reactions of the tests.

In seven instances, frog tests could not be completed because of toxicity. In none of these cases did filtration, centrifugation, or adjustment of pH reduce the toxicity, and in view of the substances which the frogs will tolerate, it seems a waste of time to treat the urine in any manner. Two specimens which were toxic to frogs caused the death of two healthy rabbits after a single 10 cc injection in each case. In one other case, urine which was toxic to frogs caused the death of a healthy rabbit after two 10 cc injections. In two instances, urine which was toxic to rabbits did not affect the frogs, and in one instance urine which was toxic to frogs was not fatally toxic for a rabbit.

When dealing with toxic specimens of urine it is often possible to complete a frog test by using fractional amounts of the urine for injections. If the patient is pregnant, the test will usually be positive if as little as one-fourth of the required volume is used. When such tests are negative, however, they should not be reported. No negative test should be considered valid unless one or more frogs have tolerated the minimal amount.

DOSAGE: The amount of urine to be injected varies with the size of the frog. It was found, after due experimentation, that most frogs will tolerate one cc of urine for each ten Grams of their body weight. That is, a frog weighing 60 Grams should be injected with 6 cc's of urine, but a frog weighing 25 Grams will tolerate only 2.5 to 3 cc's. The minimal dose is determined by ratio, and the full amount must be given in a single injection for reliable results. When frogs do not tolerate the full amount, and a negative reaction is obtained, the test should be repeated with another specimen.

METHODS FOR PERFORMING INJECTIONS: A method has been described whereby a single operator performs the injections by holding the frog by the hind legs while introducing the needle into the lymph sac.⁵ Perhaps the frogs used in this study were a little too lively, but it was not possible to duplicate this feat. When only one technologist was available an injection board (Fig. 5) was used. The head of the frog and one leg were immobilized by securing them with elastic tape, and the technologist then proceeded as if he had an assistant.

When two persons are available, the injection may be accomplished in less than one minute. The technique is as follows: An assistant holds the frogs by placing one hand around the frog's head and holding one of its legs in the other hand (Fig. 4). With the syringe held at an acute angle to the frog's leg (Fig. 4), the needle is thrust through the skin over the thigh. When the skin has been pierced, the needle is brought down parallel to the frog's leg and is directed, just under the skin, to the mid-dorsal line about one-half inch anterior to the cloaca (Fig. 5). The urine is then forced in as rapidly as moderate pressure will allow. If resistance is felt on the plunger the needle may not be in the proper place and it is best to move it until the urine flows freely. Properly injected frogs rarely move or voice disapproval, unless the urine is toxic.

ISOLATION: After injections the frogs are placed in properly labeled containers (Fig. 3). The patient's name or number, name of the physician requesting the test, time of injection, etc., may be written on the container with wax crayon.

Since frogs drink constantly (through the skin) it is best to cover the bottom of the jar or can with water. This assures a large volume of urine in the frog's bladder and facilitates catheterization.

CATHETERIZATION: This is easily accomplished by merely introducing a capillary tube into the cloaca (Fig. 6) to a depth of one-fourth to one-half inch. Urine usually flows into the tube immediately upon insertion, but occasionally it is necessary to move the catheter back and forth for two or three seconds.

MICROSCOPIC EXAMINATION: Examining the drop of urine without cover-slip has two distinct advantages: (1) The greater volume of urine provides a larger number of sperms per microscopic field in positive reactions; thus it usually eliminates the searching element; (2) in negative reactions, or when the number of sperms is small, the increased brownian movement and currents facilitate the focusing of the microscope.

There are no doubtful reactions. Spermatozoa are either present or absent, thus the test is either positive or negative.

Beginners may be confused by the presence of protozoa or bacteria in the frog's urine, but a few minutes study of the size and morphology of frog spermatozoa (Figs. 7 & 8) should make the differentiation of these organisms fairly simple. The spermatozoa in figures 7 and 8 (low power and high power views) were photographed on a standard hemacytometer mount for orientation purposes. Except for one or two organisms, only the heads are clear. In fresh mounts the tails are usually visible under low power.

REPEATED INJECTIONS: The frogs may be used repeatedly, but the number of injections to which they will respond is

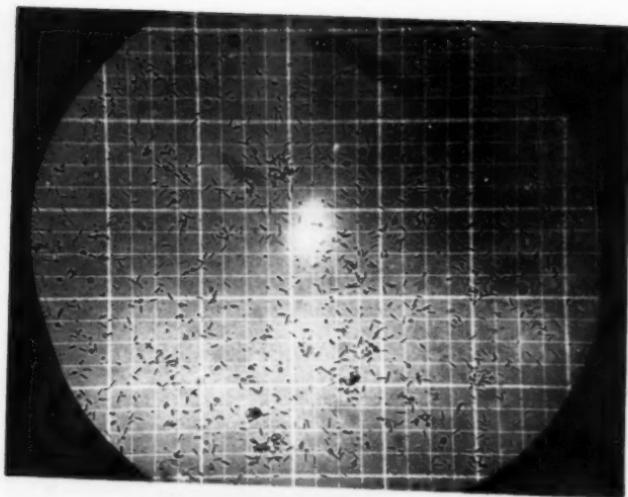


FIG. 7

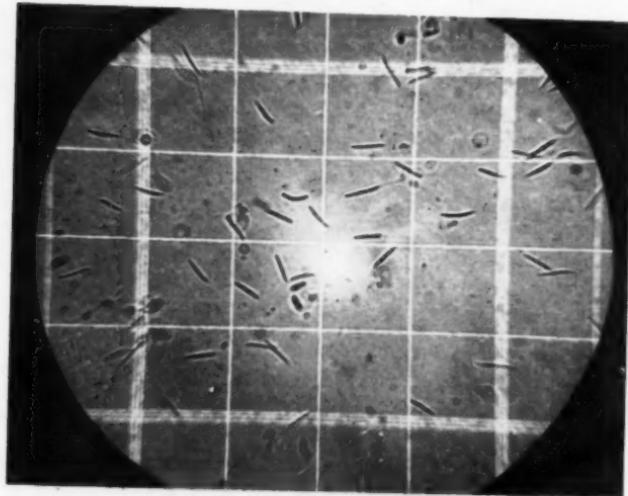


FIG. 8

probably not yet known. Twenty frogs have been injected 11 times each. During the ninth and tenth rounds of injections, some non-reacters were found, but none failed to react to the 11th injection. All of the frogs in this group have been in captivity since September. For some unknown reason, about half of them do not eat well and now appear quite emaciated. It is possible that a deficient diet was responsible for the unresponsiveness of some of the frogs. A second group of 15 frogs has been used seven times. These eat heartily and have never failed to release spermatozoa when pregnancy urine was injected.

Frogs which give a negative test may be used for another test the next day, or even the same day. The frogs which give a positive reaction should be placed in a resting tank for at least a week before being used again.

Results

ACCURACY—The reliability and limitations of this test will be discussed in several parts, and the results will be shown in tables.

Table I shows the results of a series of 112 tests, which were run in parallel by this method and the Friedman method. There were two cases of disagreement, and in both instances the frog was found to be correct and the rabbit incorrect. In addition, there were two doubtful Friedman tests. With the male frog test, there are no subjective interpretations—the test is always either positive or negative.

Table I
COMPARISON TESTS

	TESTS IN AGREEMENT		TESTS NOT IN AGREEMENT		False Negative
	Positive	Negative	False Positive	Doubtful	
Frog.....	47	61	0	0	0
Rabbit.....	47	61	2	2	0

False positive or false negative Friedman tests occur in the best laboratories. The reasons are well known, and will not be discussed here. But it might be mentioned that a report of a false positive male frog test has not yet appeared in the literature. The frog appears to be specifically sensitive to chorionic gonadotropin,⁴ and does not appear to be sexually stimulated by captive males or females; thus false positive tests seem unlikely.

The second series consists of 188 diagnostic tests, performed with frogs alone. Results appear in Table II. The one false negative reaction was obtained with an afternoon specimen. A first morning specimen from this patient, submitted the next

day, was correctly positive. It is somewhat interesting to note that this patient gave a history of previous abortion, and two weeks after these tests were performed she again aborted. Low chorionic gonadotropin levels are known to occur in abortions.¹⁴

Thirty-two other specimens collected at various times of day gave correct positive results. In 14 of these, the specific gravity was less than 1.015, and 3 specimens with specific gravities of 1.005 or less gave positive results in 30 to 60 minutes. It seems that the hormone concentration is usually sufficient to produce positive reactions at any time of day, but until more work has been reported on it would undoubtedly be wise to check all negative casual specimens with urine obtained upon arising in the morning.

Positive results have been observed with urine obtained from women whose menstrual periods were only four or five days overdue. An attempt was made to detect the pregnancy earlier, but the few women tested did not conceive during the test period.

The test was correctly positive in 3 cases of threatened abortion and one suspected miscarriage which proceeded normally after medical attention. It was also positive in one case of suspected ectopic pregnancy, but this proved to be a uterine pregnancy complicated with an ovarian cyst.

Correct negative results were obtained in the following confirmed conditions which often simulate pregnancy: Ovarian cysts, 8; uterine fibroids, 6; endometrial hyperplasia, 3; disgerminoma, 1; early menopause, 4 and psychologic amenorrhea, 2. In addition, negative results were obtained in 5 cases of miscarriage, 5 complete abortions, and 1 case of late pregnancy in which the foetus was dead.

Table II
DIAGNOSTIC FROG TESTS (Excluding Table I)

Correct Positives	Incorrect Positives	Correct Negatives	Incorrect Negatives	Total Tests	Accuracy
122.....	0	65	1	188	99.47%

The third series of tests was performed with urine obtained from patients in late pregnancy after reading reports that the test was only 50% accurate in the last trimester.⁵ The results of 41 tests on women in the fifth to ninth months of pregnancy are shown in Table III.

It has been shown by at least two groups of investigators^{14, 15} that the level of chorionic gonadotropins falls quite low in the late stages of pregnancy. Zondek states that he found his Ascheim-Zondek test unreliable in the last trimester.¹⁶ A loss of

Table III
TESTS IN LATE PREGNANCY

MONTH	5th	6th	7th	8th	9th	Total
Total Tests	8	7	8	8	10	41
Correct . . .	8	7	7	8	8	38
Incorrect . . .	0	0	1	0	2	3

accuracy may then be expected with any test in late pregnancy, but this is hardly a shortcoming.

RAPIDITY—Positive results have always occurred in two hours or less. Of 150 tests which were closely observed at various time intervals, 22% were positive in 30 minutes or less; 69% in 45 minutes or less; 87% in one hour or less, and 100% two hours after the injections.

DURATION OF REACTIONS: Spermatozoa may usually be found for 24 hours after a positive reaction has been observed. Of 25 frogs examined at various intervals, none was negative at the end of six hours; 1 was negative at the end of eight hours; 1 was negative at the end of twelve hours; 10 were negative after twenty-four hours; 21 were negative after thirty-six hours, and all were negative forty-eight hours after the injections.

The reactions may be prolonged by placing the frogs in a cold refrigerator.

Summary

A comparatively new test, employing male toads or frogs, for the laboratory diagnosis of early pregnancy is described.

The technique and performance of the test under a variety of conditions, care of the animals in health and disease and procurement and availability of animals and materials are discussed in detail.

Tables showing the comparative results of 112 tests run in parallel by this method and the Friedman method, the results of 188 diagnostic tests, and 41 tests in late pregnancy—a total of 341 tests—are included.

The results seem to provide a good basis for the conclusion that this test is the most rapid, most simple, and most economical biologic pregnancy test yet described, and that it is equal, at least, to any in accuracy.

ACKNOWLEDGMENTS

I am indebted to many people for contributions which made this study possible:

To Mr. Charles M. Bogert of the American Museum of Natural History, Dr. W. Frank Blair of the University of Texas, and Mrs. Anna Allen Wright, co-author of *Handbook of Frogs and Toads*, for furnishing much valuable information regarding amphibians;

To Dr. E. G. McCarthy, and Dr. E. O. Nichols, Jr., of the Plainview Sanitarium and Clinic, for furnishing clinical data;

To Dr. W. F. Keller, director of the Medical Arts Laboratory, Oklahoma City, Oklahoma, and his Technologist, Miss Vernal Johnson; and Dr. T. P. Churchill, director of Terrell's Laboratories, Amarillo, Texas, and his Chief Technologist, W. J. Evans, for submitting portions of urine specimens, and otherwise making possible the frog-rabbit comparison tests;

And to Thomas Locke, Evelyn Wright and Margaret McAdams for technical assistance.

REFERENCES

1. Galli Mainini, C.: Reacción diagnóstica de embarazo en la que se usa el sapo macho como animal reactivo. *Semana Médica* 54:337, 1947.
2. Galli Mainini, C.: Pregnancy test using the male toad. *J. Clin. Endocrinology* 9:653, 1947.
3. Galli Mainini, C.: Reacción diagnóstica de embarazo y acción de las gonadotrofinas en el sapo macho. *Semana Médica* 54:947, 1947.
4. Galli Mainini, C.: Pregnancy test using the male batrachia. *J.A.M.A.* 138:121, 1948.
5. Wiltberger, P. B. and D. F. Miller: The male frog *Rana pipiens*, as a new test animal for early pregnancy. *Science* 107:198, 1948.
6. Robbins, S. L. and F. Parker: The use of the male North American frog (*Rana pipiens*) in the diagnosis of pregnancy. *Endocrinology* 42:237, 1948.
7. Miller, D. F. and P. B. Wiltberger: Some peculiarities of the male frog test for early pregnancy. *Ohio J. Science* 48:89, 1948.
8. Pickett, R. F., P. B. Wiltberger and D. F. Miller: The use of the male frog test as an aid in the diagnosis of retained placental tissue. *Ohio J. Science* 48:246, 1948.
9. Silbernagel, W. M., J. B. Patterson and R. F. Pickett: The significance of bleeding in early pregnancy as evidenced by the male frog pregnancy test. *Ohio J. Science* 48:249, 1948.
10. Stejneger, L. and T. Barbour: A check list of North American amphibians and reptiles. *Mus. Comp. Zool. Bull.* Vol. 48, No. 1, 1943.
11. Wright, A. H. and A. A. Wright: *Handbook of frogs and toads*. 3rd ed., 640 p. Ithaca: Comstock Publishing Company, Inc., 1949.
12. Emerson, H. and C. Norris: Red-Leg—An infectious disease of frogs. *J. Exper. Med.* 7:32, 1905.
13. Rose, M.: Care of laboratory frogs. *Ward's Natural Science Bull.* 20:44, 1947.
14. Browne, J. S. L., J. S. Henry and E. Venning: The significance of endocrine assays in threatened and habitual abortion. *Am. J. Obst. & Gynec.* 38:927, 1939.
15. Siegler, S. S. and M. J. Fein: Studies in artificial ovulation with the hormone of pregnant mares' serum. *Am. J. Obst. & Gynec.* 38:1021, 1939.
16. Zondek, B., F. Sulman and R. Black: Hormonal test for fetal death in disturbed pregnancy. *J. A. M. A.* 136:965, 1948.

THE TECHNIQUE OF ELECTROCARDIOGRAPHY

JEROME RITTER, M.D.*

The technique of electrocardiography is obviously of interest to medical technologists. In gathering this material, however, I not only had them in mind, but the larger group of physicians and other persons who are concerned in this work—because those who are not engaged in actually making the tracings are frequently unaware of the difficulties involved and the numerous errors possible; and therefore cannot be expected to recognize technical errors when they occur. It is obvious that the number of electrocardiograms taken is increasing rapidly. The public, having been educated to understand no heart study is complete without a "cardiogram," often assumes one will be taken even in routine physical examination. Surgeons are asking for more and more preoperative tracings. And in our aging population, the percentage of patients with cardiac disease is always increasing. As a result, more machines are in use and not only are inexperienced individuals taking tracings but experienced personnel are often pressed for time and must hurry in their work. All this means that more mistakes may creep in at the same time that more reliance is being placed on the results of the record.

It was thought, therefore, that a presentation of the background of each of the steps involved in making a tracing, and a discussion of some common errors, would be both interesting and useful. The material represents routine tracings taken on patients by the students of a Medical Technology School. All the tracings were repeated, and in comparing the records the technicians were able to understand what mistakes had been made and how they should be rectified. These tracings were less than 1% of those taken—indicating how seldom mistakes occur even when inexperienced personnel are doing the work. Nevertheless, both technicians and cardiographer must realize errors can and do occur, and both should be able to recognize and identify them.

Every scientific procedure must be carried out under certain specified conditions, in order that the results obtained may be compared when the procedure is repeated. In electrocardiography it has been shown that certain extrinsic factors may influence the tracing, and unless these factors are taken into account the tracings recorded on different occasions are not strictly comparable and erroneous conclusions may be drawn from them. As will be appreciated, many variables are present. It is not often understood, however, that these include the patient. By this we do not refer to each person as having an electro-

* Cardiographer, Lincoln General Hospital, Lincoln, Nebraska.

cardiogram peculiar to him, but the fact that the same patient may produce widely different tracings under different conditions. For this reason certain standard conditions are necessary, and the cardiographer should be able to assume that the tracing was taken under the proper circumstances.

For example, the posture of the patient has a profound influence on the form of the record: in some individuals, the increased sympathetic activity in the upright position may produce changes identical to those seen in organic disease. Therefore, tracings are taken with the patient recumbent. If for any reason that cannot be done, it should be so stated on the electrocardiogram. A full meal can also produce changes in the record. Tracings should be made, if possible, a few hours after eating. The influences of fear or apprehension are quite marked. The patient, therefore, should be reassured, and the procedure explained to him—particularly that he will not be "shocked" in the process.

The tracing is the record of changes of potential associated with the heart beat which have been photographed on sensitized paper. This paper has a background of horizontal and vertical lines. The interval between each of the fine vertical lines represents a duration of four hundredths of a second; the coarser vertical lines represent two tenths of a second.

If the timer is not functioning properly, the lines will not represent these exact intervals and the cardiographer may wrongly interpret the record. Cardiographs usually have some means of checking the timer so that the interval is actually what it is assumed to be. There is no indication, however, on the record of the accuracy of the timer. Because of this, the cardiographer must assume the technician has checked the machine.

The horizontal lines, one mm. apart, represent strength of current. Each horizontal interval signifies one-tenth of a millivolt. These lines reflect the magnitude of the changes in potential which produce the electrocardiogram. They will be discussed further under standardization.

TAKING THE RECORD

Standardization:

This procedure is part of the preparation before taking the tracing. It is of great importance because the amplitude of the elements of the cardiogram is one of the factors which is measured and on which the normality or abnormality of the tracing depends. The amplitude, however, depends on the standardization of the cardiograph, and if this is not accurate, any interpretation of the tracing will be misleading. As mentioned before, the horizontal lines on the paper are 1 mm. apart. Standardization involves setting the machine in such a fashion that the string will be deflected one centimeter (ten horizontal lines) for each

millivolt of current lead into the circuit. This maneuver is repeated for each lead, thereby proving to every interpreter that that lead was properly standardized. The height of the P and T waves, QRS complexes, and the position of the S-T junction and segment may all be affected by the standardization. It is imperative, therefore, that the interpreter have before him, in each lead, proof that the machine was set properly, so that any deduction he may draw will have a firm basis. When the standardization is on each lead of the tracing it indicates the machine was correctly set, and assures the cardiographer that if abnormal values of the amplitude of the deflection are found, they represent real deviations and are not due to technical errors.

Connecting and Applying the Electrodes:

The electrodes provide the means of leading the potentials from the body to the machine, in order to produce the electrocardiogram. For the usual leads, both limb and chest, the switches are arranged so that when the electrodes are properly applied, deflections will result which conform to certain accepted standards. Before the electrodes are applied, the skin is rubbed with a specially prepared paste, which dissolves the grease on the skin and assures good contact with the electrode. If the electrode is not in proper contact with the skin, a poor tracing results. Too much paste is as bad as too little. The skin should be rubbed till it is pink and the electrode snugly fitted. One of the commonest causes of a poor definition of the string is improper application of the electrode. Another common error is transposing the electrodes on the arms. Other mistakes occur. In taking the chest leads, for instance, improper placing of the exploring electrode frequently results in error because the tracing is really not taken at the place it is assumed to be. The technologist must be thoroughly familiar with the topography of the chest and the exact location of each of the positions. It is important that the paste be applied to an area no larger than that covered by the electrode. If the paste is not localized to the area just under the electrode, it can transmit the potential of that area to adjacent areas, thereby giving false readings in other positions.

Outside Influences:

When the machine has been properly standardized and the electrodes connected correctly, there are still factors which may spoil the tracing, making it impossible to interpret accurately. Most of these adverse influences may be detected and eliminated if the technologist will have the patience and perseverance not to take the tracing unless the string is absolutely razor-sharp at the time. When it is not, there are present either somatic

tremor or A-C interference or both, and either alone will spoil the appearance of the record. The commonest causes for somatic tremor are general tenseness of the patient, incorrect application of the electrodes or paste, and an uncomfortable position of the body, especially of the arms. Also incriminated are taut or crossed cables or loose connections. A search must be made for the cause when its presence is suspected. Unless there is an obvious justification, such as a patient with Parkinson's disease, somatic tremor in a tracing is unsatisfactory and indicates careless work. In addition, a rhythmic tremor may produce a tracing simulating an arrhythmia such as flutter or fibrillation. A-C interference is more difficult to eliminate. Sometimes it can only be accomplished by pulling out every connection on a floor—and even then the machine may still be influenced. One great advantage of a battery instrument is that this annoying factor plays no part in taking the tracing. Diathermy machines, however, still will influence even that machine. However, other machines are now and will continue to be in constant use and tracings taken on them are subject to this disturbance. It is instantly obvious when the string cannot be brought to a sharp focus and is plainly vibrating. Taking a tracing under such conditions is a waste of time and equipment, because it is not fit to pass judgement on. Despite this, tracings with A-C interference are common. They mean either the operator was unaware it was present, or could not get rid of it, or did not try. Artefacts are often present in a tracing. They may be due to any movement of the patient, jarring of the machine or movement in the cables. Occasionally the artefact may resemble some real disturbance in the pattern. When the possibility of artefact is present, it is wise to repeat the tracing. Loose connections at the battery terminals are particularly apt to produce bizarre artefacts.

COMMON ERRORS:

The tracings exhibited in this paper were all delivered to the cardiographer for routine interpretation. None of the mistakes was obvious to the technologist taking or mounting the record, nor was this to be expected, since most of the records were taken by students. They illustrate the pitfalls lying in wait for the unwary or those attempting to read the records without an understanding of the technique involved. They demonstrate some of the things that can go wrong from the moment the patient encounters the machine until the tracing is mounted.

Recently there have been introduced into electrocardiography new machines which in many respects are great improvements over those which have preceded them—for instance, "direct writers." These machines eliminate the camera and paper of the old instruments. The record is written directly and comes out

of the machine while the tracing is being taken. It should be clearly understood that this machine does not simplify electrocardiography — from the point of view of the technologist or the cardiographer. It simply eliminates one step in the preparation of the record — development of the exposed photographic paper. As such it is a step forward. Unfortunately, the simplicity, plus the multiple leads possible because of the switches on the machine, will probably result in many errors, many of which may not be recognized.

In the last analysis, electrocardiography is no different from any other science which depends on a record produced by a machine. Its value depends on the precision of the instrument, the accuracy of the technologist and the experience of the interpreter. If any of these factors is faulty, the report will be of little value — in fact, it will be harmful, because improper treatment may be decided upon as a result of the erroneous conclusions, to the detriment of the patient.

Transposing the Electrodes:

This common error, which all ordinary precautions still do not eliminate, is, at the least, annoying, because it means repeating the tracing; and if it is undetected, which is also inexcusable, a highly erroneous interpretation may be made. Usually the arm leads are switched — the left arm electrode being attached to the right arm and vice versa. The connections of the galvanometer are so arranged that a positive potential at the left arm electrode produces an upward deflection, a negative a downward deflection. This is done so that the deflection in Lead I would be upward in most individuals, because the left arm is usually positive. When the electrode meant for the left arm is attached to the right arm, a downward deflection is obtained because the potential of the right arm, which reflects the potential of the interior of the heart, is negative. Lead I is, therefore, upside down. Such a connection, moreover, produces a mirror-image of the usual Einthoven triangle and Lead I is not only upside down, but Lead II is really Lead III, and Lead III, Lead II. There is one condition other than transposition of the leads that will produce such a picture — mirror-image dextrocardia, a congenital condition. This is rare, and when a tracing similar to the one under discussion results, the chances are that it was caused by error rather than dextrocardia. Still, the latter must always be considered when this type of tracing is encountered.

Electrodes in Wrong Position:

Proper placing of the electrodes in the precordial leads is a major item in the technique of taking electrocardiograms. Technologists are often at a loss to understand why so much importance is attached to this. A little explanation, however, will suffice to impress the necessity of placing the electrodes exactly on the

spot they are supposed to be. Over the years a tremendous body of knowledge has been built up determining what is normal and abnormal in these leads. The criteria which have been set up are based on observations for each lead. When a cardiographer analyzes a tracing, he has these criteria in mind. However, if a CF5 tracing is really a CF3 tracing, a normal pattern may be described as abnormal, because what is normal in the CF3 position may not be normal in the CF5 position, and vice versa. Electrocardiography has become complex and more highly developed in recent years. Cardiographers are no longer content to recognize patterns and to catalogue them. They are seeking behind the patterns, trying to explain them in terms of the forces that produce them; and since each part of the heart produces its own pattern as a result of the electrical forces within it, the corollary is true: each lead is considered to reveal what part of the heart is underneath the electrode — that is, in the unipolar leads.

If the cardiographer sees the pattern of the right ventricle in the CF5 position, he is justified in assuming either marked counterclockwise rotation of the heart or right ventricular hypertrophy is present, or both. When the technologist, however, has placed the electrode farther to the right, the conclusion of the abnormality is false, as far as actual conditions are concerned, even though true in the tracing.

Mounting the Tracing:

It may seem unreasonable to discuss the difficulties of mounting electrocardiograms, which is something one could expect a child to do properly. The point is made that here no knowledge is necessary but painstaking accuracy and a never-ceasing vigilance against carelessness. Despite every precaution, mistakes will occur. This is particularly true in institutions where inexperienced personnel performs the function, or where a hurried technologist must meet a deadline. At any rate, even such a simple thing as mounting a finished tracing may result in serious errors. This happens whether the mounting is "homemade" or whether slotted or other prepared cards are used. It will always happen unless each lead is cut separately, marked on the back, and inserted into the mounting before the next lead is cut. And even then mistakes occur. Perhaps in some cases the proper order was not followed while taking the tracing. The examples given demonstrate that such mistakes do happen and why the cardiographer must always first rule out technical errors whenever an abnormality occurs in the record before him.

Figure a

This tracing illustrates the errors of interpretation which may follow incorrect placing of the electrodes. In this record, Lead

CF5 shows a broad M-shaped QRS complex. This is abnormal in any respect, because of the broadening, slurring, and notching, but it is an unexpected pattern to find over the left ventricle, which is normally under the electrode in the CF5 position. A glance at Lead I, which usually is similar to the CF5 pattern, indicates a discrepancy is present. It is probable the pattern in CF5 represents the "transitional zone" between the two ventricles. That is further to the left than found usually, and therefore must mean rotation in a clockwise direction or an enlarged right ventricle—that is, if the electrode had been properly placed. To check on this the cardiogram was repeated, and a normal pattern was found in the correct CF5 position.

Figure b

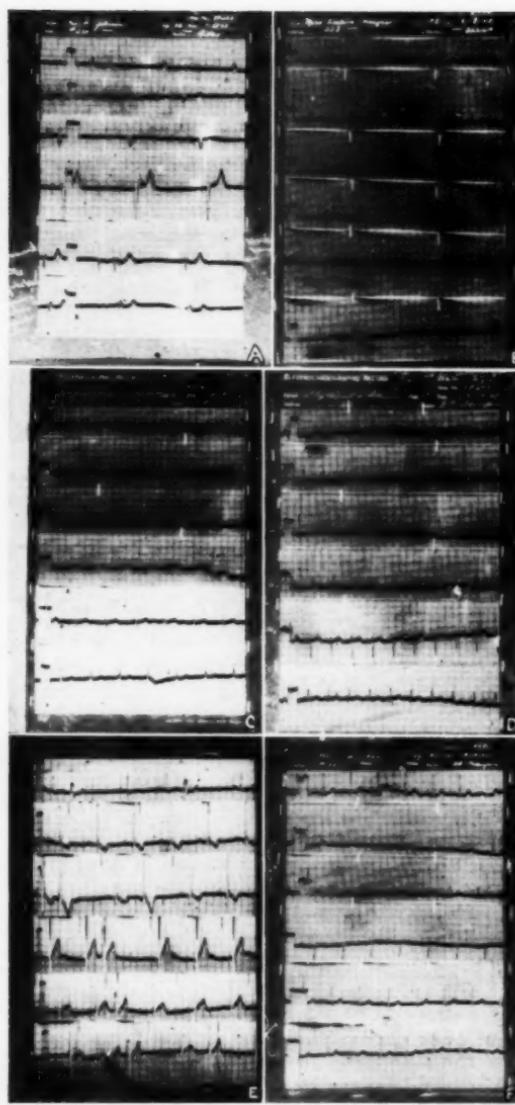
On casual inspection of this record, the single and outstanding abnormality is the low amplitude of the Lead CF4. A pronounced and unexpected drop in voltage between two normal chest leads is a highly significant finding, possibly indicative of a local disturbance in the area underlying the electrode which registered the low voltage. Such a deviation from the expected transition from one lead position to the next is perhaps even more significant than low voltage in all leads, which could easily be due to extraneous factors such as obesity. Now it is perfectly obvious that the low voltage of CF4 in this record is due to incorrect standardization, since that is included in the tracing and shows that the string moved only 2.5 mm. when one millivolt was introduced into the circuit, instead of the proper 10 mm. If the standardization had not been included or if it had escaped the attention of the interpreter, this error in technique could have led to an erroneous conclusion. This tracing also demonstrates an inconsistent standardization, since no two leads are exactly alike: in all but one the jump is less than 10 mm., and in the one not included, it is more.

Figure c

This is an instance of transposition of the electrodes. In this tracing, Lead I is obviously upside down. Lead II is really Lead III, and Lead III, Lead II. As mentioned before, a congenital mirror-image dextrocardia will give the same picture. Such a tracing must always be repeated with special attention to technique, because the dextrocardia does occur. Abbot noted 15 instances in her series of 1000 cases of congenital cardiac disease.

Figure d

This tracing demonstrates one of the odd things that can happen to trap the unwary cardiographer. On casual inspection it is a good tracing—the standards are a little off, but otherwise it is well done. The marked inversion of QRS in I and the inverted T in CF2 are the abnormalities to be noted. The former



will ordinarily be assumed to be due to right axis deviation and the latter is common finding in children. The extreme inversion of QRS in I, however, suggests the possibility of crossed arm electrodes, which, however, is ruled out by the upright P wave. The complex, therefore, must signify extreme deviation or else it is a QS and indicates myocardial damage. It is neither of these, however, because it is incompatible with CF5. Lead I almost always strongly resembles Lead CF5 because both reflect the potential of the left arm. The incompatibility raised the suspicion that some error had occurred when this record was made. To prove this it was taken again, and Lead I resembling Lead CF5 was obtained.

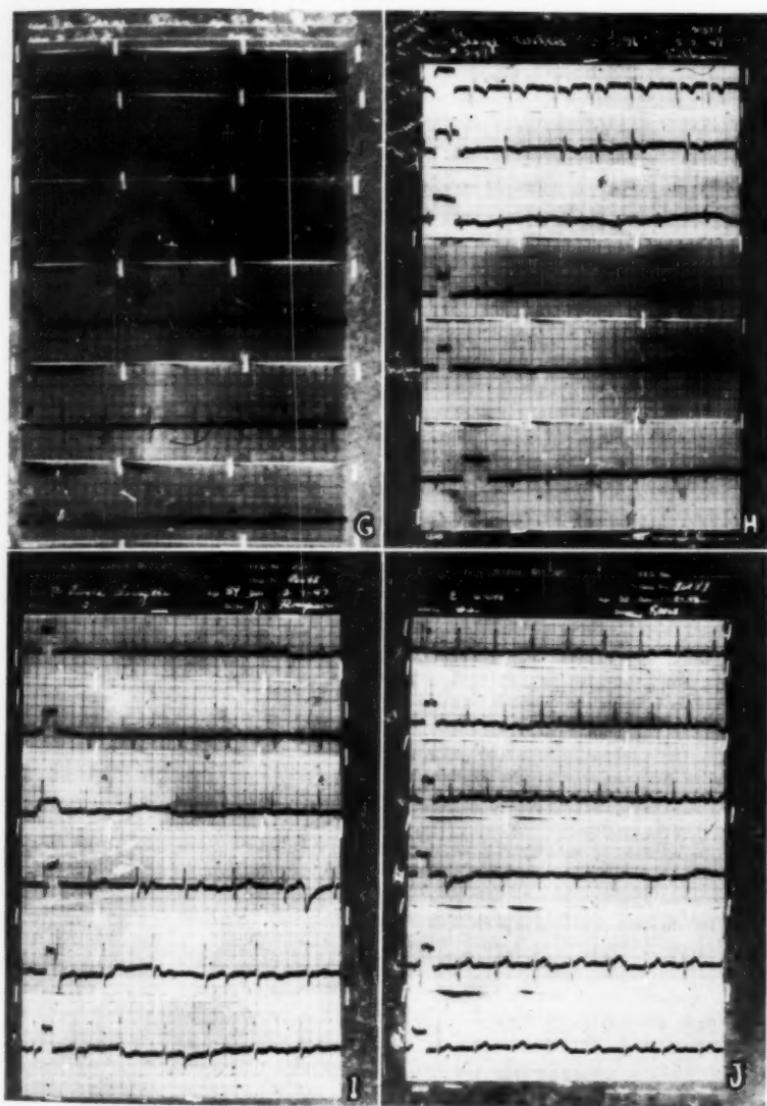


Figure e

This tracing is illustrative of the distortion that occurs when there is excessive movement of the string on introducing one millivolt of current into the circuit. The QRS complexes are tremendous and the T waves of inordinate height. These deviations however, have no clinical significance, since they are obviously due to improper standardization as is shown by glancing at the left of each lead. Again the point is made that unless each lead is standardized and the standardization included in the tracing, no conclusions can properly be drawn regarding the amplitude of the complexes.

Figure f

This tracing demonstrates an error which is hardly noticeable, and which caused some surprise when a repeat was requested, since it is as near perfect a record as it is possible to get, with only a faint A-C interference marring it. However, on close inspection it will be seen there is no difference between Leads II and III. Leads CF4 and CF5 also resemble each other. In this instance the switch must not have been turned in the first case, and the electrode not moved in the second, or not moved enough.

One must remember in taking limb lead, that the different connections are inside the machine and the different leads are obtained by the switches. This is an extremely unusual error.

Figure g

This record illustrates how the adverse influences of somatic tremor and A-C interference can spoil an otherwise good tracing and render it useless for the purpose it was taken. This patient had a first degree heart block, and the record was a follow-up, to compare his condition with what had previously been. It is impossible to measure the P-R interval here, due to the deviation produced by these outside factors. Actually, one cannot be sure of the two-hundredths of a second, which might be the difference between a normal and an abnormal record. Somatic tremor and A-C interference are frequent intruders on the electrocardiogram. They are never welcome but in most instances they do not make the differences between a useful and a useless record. The technologist, however, can never tell when such artefacts will appear, nor is he always aware when a record is being taken for comparison. Therefore, it is best not to start the camera or the paper until it is certain these factors are not operating. If the tracing is taken regardless, then this is what happens, and the record must be repeated.

Figure h

This is an example of wrong mounting. When the tracing was repeated, it was seen that the leads were actually CF2, 4, 5 and then Lead I, II, and III. This is an obvious and gross error.

Figure i

This record illustrates another instance of wrong mounting which is not obvious. Here lead II is really lead III and vice versa. In this case, the transposition was due to an error in mounting rather than crossed electrodes—the upright P waves in lead I prove that.

Figure j

This record is another example of a mixup in mounting. It is not particularly obvious and an interpreter in a hurry could easily overlook it. In this case, the lead in the CF2 position is lead III. CF5 is mounted where II should be and I where III is expected. This was proved by repeating the record.

Conclusion:

The preceding demonstration illustrates some of the errors that find their way into routine electrocardiography. Actually, such errors form a very small proportion of the total number of tracings. In fact, it is probably possible to accumulate such a "museum" only in a school for technologists, where students continue to make the same mistakes as their predecessors, and in making them, learn the technique of the science they are practicing. The responsible technologist not only knows what to do but what is behind each thing that is being done. Such a technologist will more easily achieve accuracy which is the mark of the trained worker in science.

RADIOACTIVE ISOTOPES*

By

SISTER MARY ANTONIA, S.C.N., B.S., M.T. (ASCP)

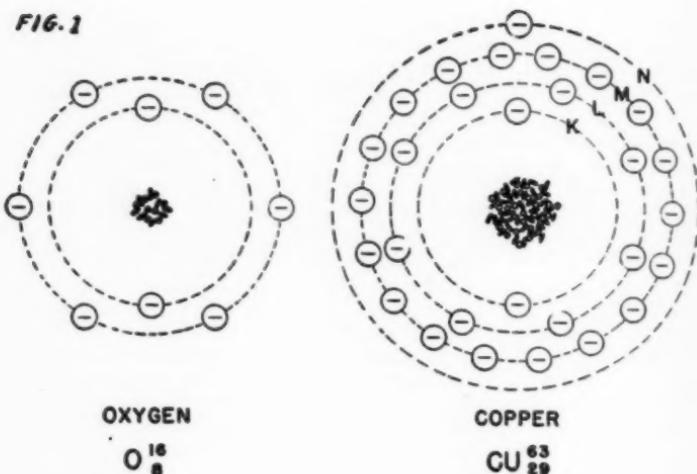
Georgetown University Hospital, Washington, D. C.

Very recently I have had the opportunity of working with men who are doing research with radioactive material. Hearing on all sides such terms as isotopes, radioactivity, pulses, tracers, Geiger Counter, I became quite interested and desired to know more about these fundamental subjects. This revelation has been so fascinating that I thought it might be to your interest to know something about this new field of radioactivity which affords such an opportunity to the medical technologists of analyzing unbelievably small quantities of material and in a minimum less time.

In order to have a better understanding of what is meant by radioactive isotopes, one needs to review the atom with its complex structure (Figure 1). Likened to our solar system with the sun as the center and the planets revolving around it in definite order, the atom has a positive nucleus for its center about which revolve electrons which are negatively charged particles. The

* Read before ASMT Convention, June 1948, St. Paul, Minn.

FIG. 1



number of electrons is in accordance with the atomic number which in turn represents the number of protons (positive charges) in the nucleus. The number of orbits which may each contain one or more electrons, may be learned from the Periodic Chart (Figure 2). The orbit closest to the nucleus is called the K orbit; next, the L orbit; then M, N, O, P, and Q.

Our interest lies chiefly with the nucleus which comprises, for the most part, the mass of the atom and is made up of both protons and neutrons (Figure 3). The protons carry the positive charge, the number determining the identity of the atom and its chemical property. The neutrons carrying no electrical charge, are electrically neutral and weigh about the same as the proton. The sum of the protons and neutrons gives the *Mass Number*. This idea is consistent with the usual chemical interpretations based on the Bohr Theory of atomic structure.⁵ On this basis, Isotopes (taken from the Greek, meaning: same place) may be described as elements with the same atomic number but a *different mass number*. To put it more clearly, the number of protons in the nucleus is the same, but there is a difference in the number of neutrons, or, that different atoms may have the same electrical charge, but variations in mass.

For example we will take hydrogen (Figure 4). Its atomic number is 1, therefore it has one proton in its nucleus and one revolving electron in the first orbit. If we were to add a neutron to the nucleus we would then have a mass twice as great but still only one positively charged particle in the nucleus and just one

PERIODIC CHART OF THE ATOMS																	
Atomic		The Atoms Grouped According to the Number of Outer Electrons								Planetary electrons in the completed shells.							
Period	Group	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	1	2	3	4	5	6	7	8
1	1	H								He	2						1
2	1	Li	Be	B	C	N	O	F		He	2	2					2
2	2	6.940	9.02	10.82	12.010	14.00	15.0000	16.183		4.033	2	2					2
3	1	Na	Mg	Al	Si	P	S	Cl	Ar	Ne	10	2	3				3
3	2	22.997	24.37	25.97	28.06	30.978	32.055	35.457	39.944	39.944	10	2	3				3
4	1	K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	18	2	3			4
4	2	39.098	40.00	45.10	47.901	50.95	52.01	54.93	55.85	56.93	58.69	18	2	3			4
5	1	Ca	Zn	Al	Ge	As	S	Br	Kr	Rb	Rb	36	2	3			5
5	2	61.554	65.45	65.55	69.77	72.96	74.91	78.96	81.97	84.92	84.92	36	2	3			5
5	3	Rb	Sr	Y	Zr	Cr	Mo	Tc	Ru	Rh	Rh	45	2	3			5
5	4	65.45	67.93	88.92	91.22	92.91	95.95	97.83	101.92	102.99	102.99	45	2	3			5
5	5	Ar	Ar	Ar	Ar	Ar	Ar	Ar	Ar	Ar	Ar	46	2	3			5
6	1	Ca	Br	La	Hf	Ta	W	Kr	Os	Pt	Pt	54	2	3			6
6	2	62.955	63.55	68.60	71.951	74.90	76.94	78.92	81.92	84.92	84.92	54	2	3			6
6	3	Ar	Ar	Ar	Ar	Ar	Ar	Ar	Ar	Ar	Ar	55	2	3			6
6	4	Ar	Ar	Ar	Ar	Ar	Ar	Ar	Ar	Ar	Ar	56	2	3			6
7	1	Fr	Ra	Ac								57	2	3			7
7	2	22.9	22.9	22.9	22.9	22.9	22.9	22.9	22.9	22.9	22.9	58	2	3			7
7	3	Fr	Ra	Ac								59	2	3			7
7	4	Fr	Ra	Ac								60	2	3			7

Fig. 2

negatively charged electron in the K orbit. Such an atom is electrically neutral, having an atomic number of 1, and a nuclear mass of 2. It is known as "heavy hydrogen" or *deuterium*. If to deuterium we were to add still another neutron to the nucleus, we are again adding to its mass number, while the atomic number remains the same: 1. This third element is known as *tritium*.⁷ The ratio of neutrons to protons in tritium, however, is too large, thus the atom is unstable and eventually reverts to deuterium or hydrogen. In this process, happening in the order of microseconds, an electron is forcibly ejected from the nucleus, thus disposing of excess mass. Unstable elements, such as tritium, are called radioactive, being entirely analogous to radium (Figure 5).

Radioactive disintegration is simply the expulsion of one small particle from the nucleus, whose other components then rearrange themselves and settle down to be (temporarily or permanently) a stable atom of another element. In naturally radioactive substances there are two particularly important types of disintegration. In one, a particle is expelled which is identical with the nucleus of a helium atom and is called an alpha particle. In the other, an electron is emitted, the result of the neutron within the nucleus breaking up into a proton and an electron. The proton left behind with its positive charge, increases the atomic number. The expelled electron is a beta particle.⁴

The particles emitted lose energy to the atoms in the near vicinity by ionizing them, that is a temporary disarrangement with

FIG. 3

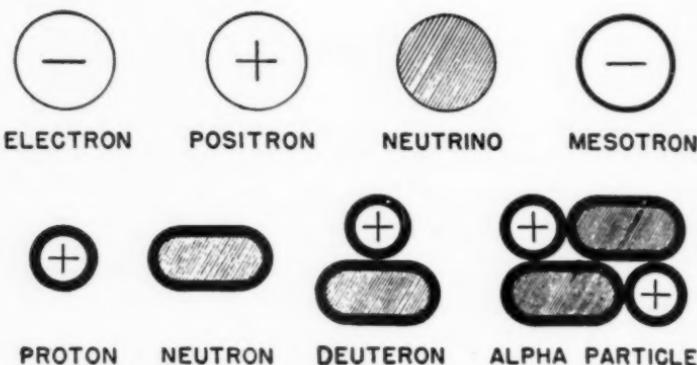
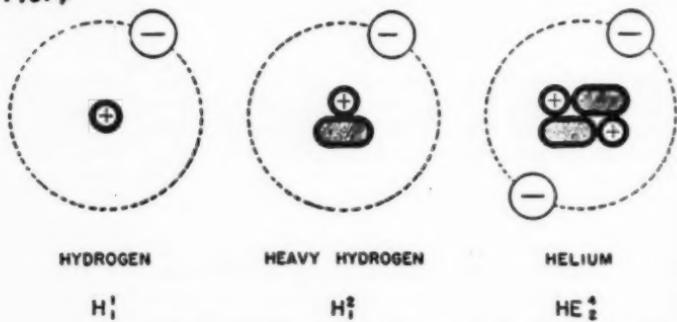


FIG. 4

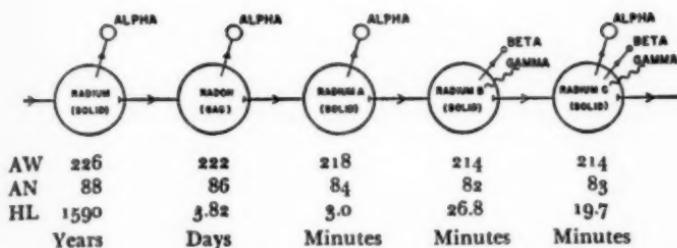


later a subsequent rearrangement of the atom to the stable form. Such an ionization may be measured and its quantity is directly proportional to the number of particles emitted. The common method of measuring this ionization, particularly in the biological field, is the Geiger-Mueller Counter (Figure 6).

In using this instrument the vapor filled G-M tube is placed near the radioactive material. By the use of high voltage which attracts the ions in the vapor to opposite electrodes before rearrangement can occur, we can detect the effect of the beta particle.

The principle may be explained in more detail as follows: As

FIG. 5



Disintegration of radium and its immediate descendants. *AW*, atomic weight; *AN*, atomic number; *HL*, half-life.

the beta particle emitted by the disintegration of the radioactive isotope, penetrates the wall of the Geiger-Mueller tube,⁹ it strikes the atoms of the organic vapor gas within the tube, ionizing the gas. The positive ions are attracted to the plate lining the wall of the tube which is attached to the ground wire; and the negative ions are attracted to the positive wire that runs through the center of the tube. By attaching the tube connections to a suitable amplifier and automatic counter, we can record the pulses. It may seem incredible but in this way we can measure with accuracy quantities of material as small as a billion-billionth of an ounce. One can easily see at a glance that much smaller amounts of material may be handled or studied by this technique than by chemical methods. Thus we have at hand a long sought-for means of studying minute amounts of substances introduced into the body.

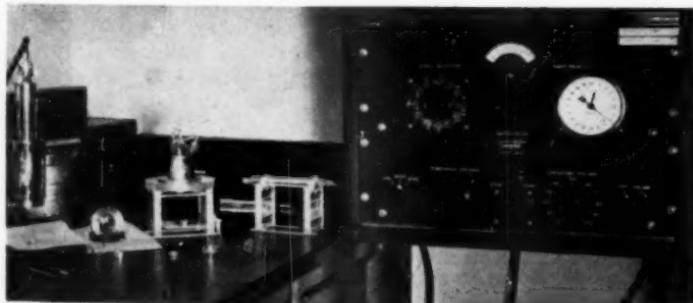


Fig. 6

It is now possible to "tag" the various constituents of a compound. For instance, in sodium chloride, either the sodium or the chloride ion may be made radioactive and traced in its passage through the biological cells. Any stable element, with which we are now working, can be made radioactive. It is possible to readily detect extremely minute traces (10^{-20} gm.) of these materials. Thus they are a powerful tool in analytical procedures.

To give you an idea of some of the work already done, we have the use of radioactive iodine in thyroid conditions,⁸ phosphorus in polycythemia rubra vera;^{1,2,3} sodium in its diffusion through extracellular fluid;^{6,10} as well as many studies in tagging of the vitamins, insulin,⁷ carbohydrates, hormones, starches, and certain drugs. One we are particularly interested in, is radioactive calcium and its use as a therapeutic agent in cancer. Report from this study will be forthcoming in the near future.

It is at this point I wish to stress the part that medical technologists play. Many tests are run on patients before, during and following therapy. A great deal of accuracy is needed to denote a slight change in the patient's condition; kidney and kidney function; blood chemistry and cytology; and the clotting mechanism are a few of the vital criteria. Much smaller samples are needed and much less time is involved in the analysis, using the new technique.

Great care must be exercised in the handling of all radioactive material, the same as any extremely poisonous, toxic substance, (1) so as not to get too great an accumulation and exceed the radiation tolerance dose to personnel, and (2) in discarding, that other materials are not contaminated.

Conclusion

In these few pages I have only scratched the surface in this field of radioactivity. Many points: such as artificially prepared radioactive isotopes, separation of stable from radioactive isotopes, mechanics of the Ionization Chamber and the Geiger Counter, explanation of the Disintegration Time or Half-life, have not been touched upon, primarily because of too great a technical involvement and secondarily because of too little time in which to present it. I hope, however, that I have made clear a few fundamental facts and have stimulated you to want to read further along this line.

REFERENCES

1. Erf, L. A.: Primary Polycythemias: Remissions Induced by Therapy With Radiophosphorus, *Blood* 1:202, 1946.
2. Erf, L. A.: Radiophosphorus as the Treatment of Choice in Primary Polycythemia, *Am. J. Med.* 1:363, 1946.
3. Erf, L. A., and Jones, H. W.: Radiophosphorus—An Agent for the Satisfactory Treatment of Polycythemia and its Associated Manifesta-

tions; A Report of a Case of Polycythemia Secondary Possibly to the Banti's Syndrome, *Ann. Int. Med.* 19:587, 1943.

- 4. Glasses, O., Quimby, E. H., Taylor, L. S., and Weathermax, J. L.: Physical Foundations of Radiology, Paul B. Hoeber, Inc., New York, 1945.
- 5. Gray, George W.: New World Picture, Little, Brown, and Company, Boston, 1936.
- 6. Kaltreiter, N., Meneely, G., Allen, J., and Bale, W. L.: Determination of the Volume of the Extra-Cellular Fluid of the Body with Radioactive Sodium, *J. Exp. Med.* 74:569, 1941.
- 7. Kamen, Martin D., Radioactive Tracers in Biology, Academic Press, Inc., New York, 1947.
- 8. Marinelli, L. D., Foote, F. W., Hill, R. F., and Hocker, A. F.: Retention of Radioactive Iodine in Thyroid Carcinomas, *Am. J. Roentgenol.* 58: 17-32, 1947.
- 9. Pollard, Ernest, and Davidson, William L.: Applied Nuclear Physics, John Wiley and Sons, Inc., New York, 1947.
- 10. Smith, B., and Quimby, E.: The Use of Radioactive Sodium as a Tracer in the Study of Peripheral Vascular Disease, *Radiology*, 45:355, 1945.

EVALUATION OF METHODS OF ENUMERATING STERNAL MARROW EOSINOPHILS

PHILIP PIZZOLATO, M.D.

From the Clinical Laboratory Service, Veterans Administration Hospital, Departments of Pathology, Charity Hospital of Louisiana, and Louisiana State University School of Medicine, New Orleans, Louisiana.

Interest has been aroused concerning the number of eosinophils in bone marrow in patients with thrombocytopenic purpuras and the part it plays in the indication for and prognosis with splenectomy.¹ In order to avoid possible misinterpretation in values of the eosinophils from technical procedures it was decided to compare the various technics recorded in the literature for the enumeration of marrow eosinophils. Since propylene glycol solution of eosin² appears superior to an acetone solution³ as a hemolytic agent in the study of eosinophils of peripheral blood, it was decided to use a propylene glycol solution of a panoptic stain along with the other technical procedures.

Materials and Methods

Fifteen patients with a variety of pathologic conditions were used in this study. One cc. of sternal marrow was aspirated and approximately 0.5 cc. was expressed gently through the aspirating needle into a bottle containing 2 mgs. of dry ammonium potassium oxalate.² From the material in the needle, slides and coverslip preparations were made and rapidly dried by waving them through the air. The oxalated material was diluted with May-Grunwald stain in propylene glycol (0.2 gms. May-Grunwald stain in 100 cc. of propylene glycol—1 cc., and distilled water pH 6.0 to 6.5—1 cc.) in a white blood pipet. Counts were

made on the Levy-Neubauer counting chamber using the 25 small central squares (0.1 cu. mm.) for counting the total nucleated elements. The eosinophils were also counted in these small squares; these cells have pinkish-yellow, red, or reddish-purple granular cytoplasm. Next the eosinophils were counted in the nine large squares (0.9 cu. mm.). Then the Fuchs-Rosenthal counting chamber was filled and the number of eosinophils was recorded in the 256 squares (32 cu. mm.). Each side of both chambers was counted.

The unused oxalated marrow was placed in a Wintrobe hematocrit tube and centrifuged at 2,000 r.p.m. for 5 minutes. Slides and coverslip preparations of the buffy layer were prepared.

The marrow remaining in the syringe was allowed to clot and then fixed in Zenker-Helly fluid. In five cases imprints of the clot were made prior to fixation. Paraffin sections were stained with Giemsa stain.

All smears were stained with Wright stain and the eosinophils per one thousand cells in duplicate preparations were enumerated.

A simultaneous count of peripheral blood leukocytes and total eosinophils were made from finger blood. The blood was diluted with May-Grunwald stain propylene glycol solution and the eosinophils were enumerated in nine squares (0.9 cu. mm.). Coverslips and slides were also prepared, stained with Wright stain, and the number of eosinophils per 200 leukocytes on each of the two preparations was tabulated (Tables 1 and 2).

STANDARDIZATION OF METHODS FOR THE DETERMINATION OF EOSINOPHILS

DIAGNOSIS	BIOCERTELLA																			
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
PERIPHERAL BLOOD																				
PERIPHERAL BLOOD	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Levy chamber	46.6	53.3	3.5	3.7	4.7	3.9	8.9	9.8	1.8	2.3	8.0	8.2	8.0	2.3	0.8	0.7	0	0	1.6	1.1
1/2 x 1/2 in. slide	54.6	57.0	4.8	2.8	7.5	6.5	12.5	17.0	3.0	3.0	4.9	3.0	8.0	2.0	2.0	0.8	0	0	1.6	2.8
22 cu. mm. coverslip	37.0	33.6	1.5	2.9	4.8	8.0	14.9	13.5	4.0	2.0	8.0	8.0	4.8	2.0	0.0	0.0	0	0	1.8	1.9
E.S. per cu. mm.	19,800	15,900	14,700	38,100	38,300	38,300	38,300	38,300	38,300	38,300	38,300	38,300	38,300	38,300	38,300	38,300	38,300	38,300	38,300	38,300
Levy chamber	48.3	55.4	0.8	3.1	2.0	3.8	not done	1.7	0.4	3.0	4.8	2.6	4.9	0.7	1.6	1.7	2.9	0.3	1.9	
1/2 cu. mm.	47.5	48.1	1.8	2.3	2.4	3.6	4.7	4.5	1.8	0.8	3.9	3.7	2.8	3.3	1.1	0.9	2.0	2.0	1.8	1.8
Levy chamber	47.5	48.1	1.8	2.3	2.4	3.6	4.7	4.5	1.8	0.8	3.9	3.7	2.8	3.3	1.1	0.9	2.0	2.0	1.8	1.8
Fuchs-Rosenthal	50.1	44.1	2.8	2.5	2.1	7.8	4.0	4.9	1.8	1.1	4.0	3.8	2.1	2.9	0.9	0.8	0.7	2.4	0.1	1.8
1/2 cu. mm.	50.1	44.1	2.8	2.5	2.1	7.8	4.0	4.9	1.8	1.1	4.0	3.8	2.1	2.9	0.9	0.8	0.7	2.4	0.1	1.8
1/2 cu. mm. slide	50.1	44.1	2.8	2.5	2.1	7.8	4.0	4.9	1.8	1.1	4.0	3.8	2.1	2.9	0.9	0.8	0.7	2.4	0.1	1.8
22 cu. mm.	50.9	50.7	1.8	1.8	2.8	4.6	3.0	0.2	2.0	0.8	4.0	4.7	2.1	2.6	1.9	0.9	1.8	1.1	1.7	1.1
22 cu. mm. coverslip	48.9	52.1	2.8	1.6	4.6	3.6	0.9	4.3	0.8	1.9	4.1	3.6	2.7	2.5	1.8	2.1	2.2	2.3	2.1	2.0
Levy chamber	40.0	40.0	1.7	1.7	2.9	4.9	12.5	7.8	1.3	1.8	4.1	2.1	2.8	2.0	not done	1.0	2.0	3.1	2.0	
1/2 cu. mm.	41.7	33.9	1.7	2.8	3.0	4.6	7.6	7.3	0.8	0.6	3.4	3.7	3.4	3.0	0.8	0.6	2.3	2.0	3.4	2.8
Levy chamber	41.7	33.9	1.7	2.8	3.0	4.6	7.6	7.3	0.8	0.6	3.4	3.7	3.4	3.0	0.8	0.6	2.3	2.0	3.4	2.8
Levy chamber	51.9	52.7	2.8	5.3	4.8	2.4	0.5	0.3	1.0	1.9	0.9	0.2	4.8	3.0	2.7	1.8	2.5	2.2	0.2	0.5

EVALUATION OF METHODS FOR THE ENUMERATION OF EOSINOPHILS

DIAGNOSIS	DETERMINATION METHODS	AGANZO-CYTOSIS		HYPOCRONIC ANEMIA OF PREGNANCY		HYPOCRONIC ANEMIA OF PREGNANCY		HODGKIN'S DISEASE		PACETS DISEASE	
		1	2	1	2	1	2	1	2	1	2
PERIPHERAL BLOOD	EOSINOPHILS IN PERCENT										
	Levy chamber										
	0.9 cu.mm. 3 x 1 inch	5.8	5.1	0.3	0.0	3.9	2.2	2.3	1.5	1.1	0.6
	slide	2.5	4.0	0.5	1.5	3.5	3.5	2.0	1.0	1.0	1.0
	22 sq.mm. cover-slip	3.0	3.0	0.0	0.5	3.0	1.0	2.5	2.5	1.0	2.0
	T.N.E. per cu.mm.	87,200		140,000		98,600		179,450		88,450	
STERNAL MARROW	EOSINOPHILS IN PERCENT										
	Levy chamber										
	0.1 cu.mm.	3.9	4.7	2.3	2.4	2.8	2.2	3.7	4.4	1.8	1.8
	Levy chamber										
	0.9 cu.mm.	3.8	3.4	1.6	1.6	1.9	2.3	3.2	4.1	2.3	2.1
	Fuchs-Rosenthal chamber										
	3.2 cu.mm. 3 x 1 inch slide	3.8	3.9	1.5	1.5	1.8	1.9	3.9	3.6	2.2	2.1
	direct	3.9	2.4	0.9	0.8	1.5	3.2	3.3	3.9	2.1	1.4
	22 sq.mm. cover-slip										
	direct	4.5	4.5	1.1	1.1	2.3	1.5	3.2	3.9	2.4	2.1
	3 x 1 inch slide buffy layer	4.3	3.3	not done		3.3	2.9	3.3	2.9	1.4	2.1
	22 sq.mm. cover-slip buffy layer	4.9	3.4	1.3	1.6	2.9	3.4	1.6	2.7	2.1	2.3
	Histologic section	6.8	6.5	2.3	2.1	4.1	5.0	5.7	6.1	3.6	3.4
	Imprint	2.0	2.6	2.4	2.3	1.5	2.9	3.1	3.6	1.1	1.1

Discussion

In each case the results obtained from a single procedure as well as from multiple procedures showed variation. From the data available the author is not prepared to state which method is the most accurate. There is a strong impression that the reliability of any single procedure is directly proportional to the total number of cells counted. However, a practical solution to the problems of counting cells must be found involving simplicity with the least compromise of accuracy.

There are advantages and disadvantages to all methods. The chamber method causes the least trauma to the cells and gives a uniform distribution. The larger the area counted the more constant are the results. Megakaryocytes can be counted simultaneously. The disadvantages of the chambers are the necessity

for making the counts immediately and the loss of fine cellular detail.

Smears give the best cytological detail but crushing of many cells is an objectionable feature. There is no significant difference in results obtained with either slide or coverslip preparation. However, the slide is simpler to prepare than the coverslip. The use of smears of the buffy layer is advantageous in hypoplastic marrows.

Paraffin sections offer no advantages and involved the most technical preparation. Generally higher values appear to be obtained with this method than with the others. Although imprints were prepared from the same clot on which subsequent paraffin sections were made, the imprint counts were uniformly lower than the section counts. This suggests that eosinophils may possess poor "stickability."

Conclusions

The Levy Neubauer chamber method with the use of May-Grunwald propylene glycol stain plus any of the direct smear methods are suggested for the enumerating of eosinophils in marrow. The chamber, besides offering a fairly uniform distribution of eosinophils gives the total number of nucleated cells and megakaryocytes.³ The smear is recommended for fine cytological detail.

From the data presented it is important that one must use reservation in interpreting or comparing results of differential counts on marrow especially when a small number of cells is enumerated.

REFERENCES

1. Discombe, G.: Criteria of eosinophils. *Lancet* 1: 195-196, 1946.
2. Pizzolato, P.: Sternal marrow in health and disease. *New Orleans M. and S. J.* 100: 3-6, 1947.
3. Pizzolato, P.: Sternal marrow megakaryocytes in health and disease. *Am. J. Clin. Path.* 8:891-897, 1948.
4. Randolph, T.: Enumeration and differentiation of leukocytes in counting chamber with propylene glycol-aqueous stains. *Proc. Soc. Exper. Biol. and Med.* 52: 20-24, 1943.
5. Schwartz, S. O.: Prognostic value of marrow eosinophils in thrombocytopenic purpura. *Am. J. Med. Sci.* 209: 579-587, 1945.

WHY WRITE A SCIENTIFIC PAPER

MISS ESTHER I. WILBRECHT, *New Ulm, Minnesota*

Once or twice during the year letters are sent to members of this organization requesting the individual to present a scientific paper, the subject to be of his or her own choosing. Many of these requests are immediately disregarded. The program chairman has nothing tangible on which to build up his program until the so-called deadline for the expected arrival of papers approaches. This method of collecting scientific papers has always seemed haphazard and hopeless. Perhaps it would be wiser if specific subjects were delegated to certain individuals; but this also has its drawbacks. There are many members who feel they do not have a talent for creative writing. It is to these individuals this paper is directed.

In the writing of a scientific paper, the first question the author should ask himself is, why do I want to write a scientific paper? Is it because I think it is a part of my duty in the practice of my profession or its specialties, and will the writing of this paper broaden my knowledge of the particular subject on which I am writing? Is it written as a result of an invitation from the chairman of the program committee or the secretary of the District, State or National Society? Am I being asked to write because of some particular knowledge that I have as a result of clinical experience beyond those of others? When we consider how little is known regarding the writing and presentation of scientific papers, Doctor Richard M. Hewitt's description and outline can be appreciated in the light of subsequent detailed studies. The most significant observation made by Doctor Hewitt appears to be this, that individual authors recognize the importance of using terms and expressions which are standard in the field and thus will be understood exactly by the readers.

The type of paper written should vary with the answer to these questions. In other words, we will take it for granted that the paper will follow the accepted style of presentation, that it will contain material which would be fitting for the society to which it is to be presented or the scientific journal to which it is to be submitted. For example—The report of an interesting case would be a good type of presentation for a local staff meeting of a district society meeting; it would not be a particularly good presentation for a State and most certainly not for a National meeting. On the other hand a series of clinical observations or experimental ones, if briefly presented, and especially if the

experimental data has clinical application, could be presented before all groups referred to above.

The simplest scientific paper for the beginner to write is one in which an interesting case is reported. It would be well to search the literature to see whether or not the case is a rare one and also whether or not in his presentation the writer could contribute something to the scientific knowledge dealing with such cases. In this phase it would deal with newer or modified technics.

The second classification includes those which give a report of clinical observations or experimental studies. Since it is almost impossible to read all the published work on even the smallest aspect of the medical field, it is therefore more important than ever to note the titles, conclusions or the summaries. This should be constantly kept in mind for those who desire to write on a scientific subject. It is said that the greatest value in the writing of a scientific paper is to the person who writes the paper.

It is also advisable that the inexperienced writer adhere to the form of scientific writing which is being used by the best scientists of the day. The particular style which anyone may develop will come only with experience, and all of us must be constantly mindful of the fact that what may have been our style of writing in the past may be very bad style. Then we must recognize it as such and do everything we possibly can to improve it and to develop a style which is interesting and in conformity with the best writing of the time.

Since we wish to adhere rather closely to the problem of the writing of scientific papers, it would not be out of order to quote from Mr. H. L. Mencken's essay written many years ago. "When we read particularly interesting sentences which carry valuable information, when the sentences are well constructed and the words are well chosen, we must not get the idea that the sentences were written off on the spur of the moment by the writer." As Mencken said, "writing sentences of that sort as a part of an article or paper can only be done with good, honest work in which the structure of every sentence and the words used therein are carefully chosen." There are few good writers who can sit down and dash off a fine, well-written, informative article or a story in an initial copy. The world famous Doctor Osler emphasized the fact that he made at least eight drafts of every paper that he wrote.

The following outline prepared by the American Medical Writers' Association, and discussed by Doctor Arkell M. Vaughn at the Fifth Annual Convention of that Association, is the usual and most acceptable method to follow when preparing a paper for presentation.

GENERAL OUTLINE

1. *Introduction*

The introduction should be the first paragraph. In the introduction the writer should inform the reader what the author is going to discuss and why, in his opinion, the subject is important enough to warrant a paper. This may be a new procedure, technic, chemical analysis, or such work as would come under his or her jurisdiction.

2. *Review of the Literature*

A short and concise review of the literature should then follow in an orderly and chronological manner. This review will indicate to the reader the amount of study which the writer has given this subject.

3. *Methods or Technic*

The methods or technic employed should then be given if a new procedure is being described. This should be very clear, concise and easy to read.

4. *Results*

The results obtained should be clearly given. Tables, charts, photographs and diagrammatic drawings will many times clarify the results. The tables should be in bold print and should not contain too much material. Many times the reader is discouraged by, and will not read, charts or tables which contain a large amount of material which is written in fine print.

5. *Discussion*

A discussion should follow the results and the pertinent points elaborated. Some writers write profusely at this point and many times stray from their subject with non-pertinent material, thereby making the article exceptionally wordy. A thorough discussion is important, however, if the author stays within bounds.

6. *Conclusions*

The conclusions bring out in a short paragraph or two the main points of the paper and attempt to prove what the author originally intended to convey.

7. *Summary*

In the summary all the important points of the paper should be summed up 1, 2, 3, order, so the reader can grasp the high points of the paper and decide whether or not he desires to read the entire article.

8. *Bibliography*

When a long bibliography is noted at the end of a paper, it

signifies that the writer has spent many hours of valuable time in compiling his paper. Due consideration should be given to the authors, title and name of journal.

9. *Grammatical Construction*

A paper should read easily, be understandable and be as free as possible from grammatical errors. Doctor Waltham Walters of the Mayo Foundation stresses the point that a paper should be written and rewritten, read and reread before it is submitted to the editors for publication and should be submitted in the best possible form. Even, then, after several readings, a misspelled word may show up in the galley proof.

Lastly, an author should not forget that when he sends a paper in for publication, this should be the final draft of the paper. When the galley proof is sent to him, it should be gone over quickly and carefully for typographical errors, but words or sentences should not be rearranged unless they have been erroneously printed. The galley proof should be returned without delay. There is nothing which will increase cost of publication more than to have changes made in the text of an article after the type has been set and the author has received the galley proof. Authors who persistently follow the practice of making changes in their manuscript after they have received the galley proof of it, become so unpopular that many times editors will refuse to accept their articles, no matter how valuable the information they contain. Especial care should be given to the first paragraph of the paper and the summary in order to invite a thorough reading of the whole paper.

Before closing it would not be amiss to quote from Doctor Richard M. Hewitt's lecture "Organization and Outline of Paper" as used at the Mayo Foundation. A device was worked out in the construction of a certain book of about 350 pages. Two outlines were used. The first was a list of chapter headings typed on a sheet of paper. This outline was general. By reference to it, whenever the author had become engrossed in one portion of his task and did not remember the chapter which he was writing, he could glance at the list of chapter headings and become oriented again.

Next the card outline was made. It was detailed. Therein nearly every thought, illustration, and reference was recorded until, finally, two boxes were full of cards. Individual cards and batches of cards could be shifted or discarded. Additions and deletions could be made. Everything was fluid until the last moment. On some cards appeared sentences that eventually appeared in the book; on other cards were only notes; on still others, abstracts of articles. The author was not confined by the

form that he had adopted; he did not become a slave to his system; he remained the master of it.

The value of the method was that all the thinking had been done before the author began to write. He could write as much as a card indicated, turn it down and forget it. Each time he sat down to write he did not have to attempt to read all he had written before in order to see where he was, a task that becomes impossible as bulk of manuscript increases. Almost all that he had to pay attention to was the mechanics of writing. The result was a well-planned book and an author, secretary and editorial workers who were not exhausted when the book was done.

Customarily, nowadays, a paper ends with a section headed "Conclusions," "Summary" or "Summary and Conclusions," again, there may be two sections, one headed "Summary" and the other headed "Conclusions." The terms are not interchangeable. Conclusions are general statements of truths established in the paper and, of course, they are not warranted unless the paper is conclusive. One style is to print them in short, numbered paragraphs, although the form is of small importance compared to the content. A summary merely recapitulates what is in the paper. It may contain anything that is brought out in the body of the paper. It should not contain new thoughts or new facts. If it does contain them, it is evidence that the paper was not well constructed because apparently, the author had not finished his paper before he began to summarize. One style is to run the sentences of a summary, unnumbered, one after the other, in a continuous paragraph; occasionally more than one paragraph is needed.

In summarizing the foregoing outlines I hope that I have been successful in assisting those who are timid and feel that their work is not acceptable for public consumption, yet have the opportunity and talent for producing scientific papers of unusual merit.

BIBLIOGRAPHY

1. Hewitt, Dr. Richard M., Writing and Presentation of Medical Papers: Some Suggestions. *Miss Valley Med. Jr.*, Vol. 71, 22-25, 1949.
2. Walters, Dr. Waltman, Writing Papers on Surgical Subjects, Lecture, Fifth Annual Conv. Am. Med. Writers' Assoc. *Miss. Valley Med. Jr.*, Vol. 71, 26-29, 1949.
3. Vaughn, Dr. Arkell M., Discussion of Doctor Waltman Walters Lecture. *Miss. Valley Med. Jr.*, Vol. 71, 29-31, 1949.
4. Fishbein, Dr. Morris: *Medical Writing, The Technic and The Art*. Second Edition, 1948.

NEW PATHS TO PEACE THROUGH UNESCO*

By BELLE BOONE BEARD

Professor of Sociology, Sweet Briar College

My title might well be: "The You in UNESCO" because I am going to speak about what UNESCO means to you and what you mean to UNESCO. But first, I want to ask you to repeat aloud with me that poignant sentence from the UNESCO constitution: "Since wars are made in the minds of men, it is in the minds of men that the defenses of peace must be constructed."

UNESCO means a framework within which each one of you who sincerely wishes peace may work for its achievement. The purpose of UNESCO is "to solidify and give universal meaning to the friendly impulses" of men and women the world over. UNESCO is formed on the belief that peace can be achieved not through force but through understanding. As Dr. Milton Eisenhower has pointed out: "We do not believe that understanding alone will bring peace but it is an indispensable condition of peace. There is no hope that we will ever have permanent peace until there is understanding and friendship among the peoples of the world."

The U. S. Congress, when this country first became a member of UNESCO, provided for a National Commission. This body—the U. S. National Commission for UNESCO—has a double responsibility. Made up as it is of 100 distinguished men and women, forty selected as individuals, sixty from national organizations, it acts in an advisory capacity to the State Department on matters pertaining to UNESCO. It also is charged with the duty of carrying the UNESCO program to the people of this country. Among its members are scientists, educators, economists, editors, industrialists, creative artists, and government officials, who give generously of their time and abilities in pursuance of these tasks. Similar groups are established in other lands as part of this great movement whereby "peoples speak to peoples," and the learning of the specialist may be put at the service of the man in the street.

Perhaps we are in the habit of thinking of War and Peace being made by forces remote from ourselves—by governments or by diplomats. If we agree with the fundamental principle of UNESCO namely that Peace is made in the minds and hearts of men then we must realize that the groundwork for peace is carried on not only by official representatives in striped pants, but also by MD's and MT's in white coats, by farmers in blue overalls, by white collar workers, by professors in black robes, and by members of labor unions. "The gap which has so often existed between foreign offices and the people they presume to

* Given before American Society of Medical Technologists, Roanoke, Va., 6/23/49.

represent will be much narrowed, and perhaps closed. A great instrument will have been forged for the achievement of UNESCO's dream—Peoples talking to peoples directly, discussing mutual problems, collaborating on the same projects, and eventually understanding each other so completely that no lies told about each other can be persuasive." Think, for example, what it would mean to the peace of the world, if all the Medical Technologists in all lands understood and trusted each other. UNESCO provides a clearing house for all organizations working for peace through tolerance and understanding. UNESCO does not try to supersede any organization or to take over the functions performed by any group. Rather it seeks to facilitate the peace making efforts of existing agencies.

UNESCO embodies the oldest known formulae for peace yet is a new social invention. Its principles of mutual aid and co-operation reflect fundamental religious and philosophical creeds which have survived countless ages. Yet the plans for helping people achieve these noble aims are practical and modern. They involve the latest inventions of communication: radio, newspapers, airplane and microfilm. They utilize new techniques devised by social scientists such as methods of measuring and interpreting social attitudes, social schemes for exchange of information and personnel, new methods of conducting conferences and cooperative projects; ways of discovering and alleviating group tensions, means of detecting and eliminating personal prejudices. In other words, UNESCO provides the framework within which individuals and groups can establish personal relations with similar individuals and groups in other lands.

One of the basic principles of UNESCO is that of shared experience. It is the opposite of exclusiveness, of isolationism. I want to emphasize this point particularly to you. You are a group of experts and the expert often gains his status and his public recognition from his body of exclusive knowledges or skills. The expert is different, is someone set apart by his superior accomplishments. I am afraid that in the past, the American expert has been more concerned with the exploiting of his superior position for financial gain or for renown than he has with sharing his expert "know-how" or in trying to understand, appreciate, and help the experts of other lands.

I have been concerned, as I have talked to young engineers, doctors, machinists, psychologists, salesmen and advertising men who are being trained for professional jobs in foreign countries. They seem, by and large, to be thinking about the money they will make, the high position they will have as a result of their superior training. When there is mention, as there sometimes is, of the expert service they wish to render to other nations it is seldom in terms of shared knowledge for the benefit of man-

kind but in making available for a price an exclusive possession, which can and will be discontinued at the will of the expert. Is it any wonder that most of the people of the world look upon Americans with suspicion and fear? It is experts such as you who can change that attitude.

In a world still emotionally shattered, still recoiling from almost unendurable experiences, still rancored with hatred and suspicion, efforts at the building of peace proceed slowly. The only encouraging fact is that human beings seem to possess an imperishable faith which enables them to respond to personal kindness. Men and women of whatever class, color or creed have similar fundamental yearnings:

1. to be respected as persons
2. to be treated with dignity and kindness
3. to share in the worthwhile work of the world
4. to have a sense of belonging to congenial groups.

It is one thing to publicly approve the program of UNESCO; it is quite another to put it into operation in one's own life. Even if we honestly believed that we could foster peace by giving mutual respect and help to fellow experts, are we willing to pay the price? Are we courageous enough to overcome our prejudices in order to teach, to work with, and to learn from people of other classes, colors and creeds? I believe you will agree with me that any national professional organization which permits any discrimination because of color or creed is a menace to world peace.

I hope that before another convention you will have organized an International Society of Medical Technologists and that each division or branch will have under way at least one project, operating under the guidance of UNESCO. You may want to develop an extensive plan such as the American Dental Association has engaged in for several years which includes aid in educational reconstruction, exchange professors, joint conferences, etc. Or you may want to participate as smaller units in offering scholarships for persons from Greece, Finland, or Peru to be trained in our splendid institutions; or to organize a good will tour for members from your own organization to visit France and Italy to meet Medical Technologists in those lands, to discover their needs and to offer assistance and encouragement. Smaller plans may involve exchange of scientific publications and gifts of much needed apparatus, instruments and drugs.

But let us always bear in mind that as educators do not necessarily educate for peace neither are Medical Technologists automatically emissaries of good will. In choosing representatives to send to other lands choose persons known to be good in their specialization but also known to be interested in human beings and in building the defenses of peace.

UNESCO offers you an opportunity to do your bit toward the establishment of peace. Are you willing to accept the challenge?

THE PRESIDENT'S PAGE

Dear ASMT Members,

Another convention is now history, and we start a new year working to make even greater progress to report at our eighteenth annual convention in Houston. Reports of the seventeenth convention held at Roanoke, Virginia, will be found on other pages of this Journal.

This year we are going "all out" for a membership drive. The Membership Committee solicits the cooperation of all membership committees in every state to cooperate in making this a really big year in an increased membership. And to prove they are interested in more members, your membership committee seeks to make every member—old or new—an ACTIVE member in its strictest sense.

The biggest news we have for you as this Journal goes to press is the appointment of a full time Executive Secretary-Editor-in-Chief of AJMT. Miss Rose Matthaei, whom you have all learned to know through our very excellent Journal, has been appointed to this new post. Our sincere thanks to Hermine Tate for her service to ASMT as Executive Secretary.

FOLLOW YOUR JOURNAL CAREFULLY FOR NOTICES REGARDING A CHANGE OF ADDRESS OF THE EXECUTIVE OFFICE. Give Rose your support in getting this new office started! We'll all visit the Executive Office next June—so Rose, get out the welcome mat!

Sincerely yours,

Ida L. Reilly, M.T. (ASCP)
President ASMT.

**NOTES FROM THE 17th ANNUAL CONVENTION
OF THE AMERICAN SOCIETY OF
MEDICAL TECHNOLOGISTS***

Some 429 persons, representing 41 states and the District of Columbia, registered for the seventeenth annual convention of the American Society of Medical Technologists in Roanoke, Virginia. As of the April 30, 1949, audit, there are 3693 members of A.S.M.T. in good standing.

The Reception on June 19, at the Hotel Roanoke was well attended, and gave those of us who were fortunate enough to be there a day early a good opportunity to renew old, and make new, acquaintances. The doctors of Roanoke proved their mettle

*Report from the House of Delegates session will be given in more detail in a later issue of the Journal. A copy of the Minutes had not been received in the Editorial Office in time to be included in this number.—Editor.

in the drives around scenic Roanoke, and to the top of Mill Mountain. Roanoke has its full share of beautiful surroundings, and its full quota of the ability to dispense the proverbial "Southern Hospitality."

The Advisory Council meeting again proved itself of inestimable value as the ground for discussion of matters which concern all of us in medical technology. One of the best developments of this session was the motion to establish a Public Relations Committee which will assist in making known to the public the position of Medical Technology in the field of Medicine.

An outgrowth of the Advisory Council meeting was an evening session open to the membership. Dr. Montgomery, Chairman of the Board of Registry, and Mrs. Ruth Drummond, Registrar, replied to a number of questions which have arisen from time to time when medical technologists have given thought to the many problems of medical technology, especially those which arise from the relationship of those practicing a profession to their certifying board.

HILLKOWITZ MEMORIAL AWARD

At the Dogwood Breakfast the first Hillkowitz Memorial Award was presented to Mr. John A. Mooty of Wood, Wisconsin, for his paper on "The Effect of the Age of Serum Samples and of Alimentary Lipemia on the Thymol Turbidity Test". We are happy to announce that the Denver Chemical Manufacturing Company and the Will Corporation who so generously sponsored this award, have announced their intention to sponsor the second Hillkowitz Memorial Contest award of \$200.00 for 1950. Many of you will, no doubt, plan to submit your papers so watch subsequent issues of the Journal for detailed rules.

At the meeting of the House of Delegates the following were elected to office: President elect: Vernal Johnson, Oklahoma City, Oklahoma; Secretary: Sister Eugene Marie (Carpe), Cincinnati, Ohio; Board of Directors: Grace Ballard, Milwaukee, Wisconsin; Anne J. Sommer, St. Louis, Missouri.

Houston, Texas, was selected as the convention city for June 1950. Boston, Massachusetts, was selected as the convention city for 1951.

The Plantation Dinner was an added highlight of the convention. Ordinarily it could have passed muster as the official banquet.

At the annual dinner, the Colonial Banquet, the Awards Committee announced that Mr. L. B. Soucy, Plainview, Texas, had received the individual award for his exhibit of "Pregnancy Test Using American Frogs and Toads," and that the Colorado So-

society of Medical Technologists again had received the state award. First award for her paper on "The Preparation and Use of the Rh Hapten," went to Miss Betty Brockland, St. Louis, Missouri. Mr. Soucy received the second award for his paper on "The Use of Ordinary Toads and Frogs for Pregnancy Tests." Mr. John Frazer, Vicksburg, Mississippi, received the third award for his paper on "Circulating Blood Volumes in the Laboratory." Honorable Mention was given the paper on "Determination of Rh-Hr, CDE-cde Antigens and Antibodies," by Ruth Guy, of Dallas, Texas.

Much credit should be given to all those who made this seventeenth annual convention such a success, especially to the General Chairman, Miss Ida Reilly, of Roanoke, to the members of the Virginia Society and the "neighbors" who served on the various convention committees, to Miss Mary Eichman, and members of the Program Committee, to Miss Doris Boon and her assistants who gathered together an outstanding group of scientific exhibits*** and to Miss Cecelia Kortuem, whose efforts with the Technical Exhibit section were manifest in the quality of those exhibits.

*** Scientific Exhibits:

- "Pregnancy Test Using American Frogs and Toads."—L. B. Soucy.
- "The Cytologic Diagnosis of Cancer."—Phyllis Stanley, Division of Cancer Control, N. J. State Health Dept.
- "Large Paraffin Sections for Lantern Slide Projection."—Miss Alvina Henning, Fitzsimons General Hospital, Denver, Colorado.
- "Amoebiasis in Wisconsin."—Mr. John A. Mooy and Andrew Orlander.
- "M. Tuberculosis."—Maxine Feltz and Isabella Fraser, Colorado.
- "Histoplasmosis."—R. C. Rogers, M.D. and Kathryn F. Fean.
- "The Preparation and Assay of Rh Hapten."—Dorothy Fengler, Chas. T. Miller Hospital, St. Paul, Minn.
- "Identification of Yeastlike Fungi from the Vagina."—Mrs. Louise Cason, Medical College of Alabama.
- "The Training of the Registered Medical Technologist in Michigan," by The Michigan Society of Medical Technologists.
- "Activities of Colorado State Society of Medical Technologists," by Colorado State Society of Medical Technologists.
- "1849, One Hundred Years of Progress, 1949," by Minnesota Society of Medical Technologists.
- "The Growth and Development of Medical Technology in West Virginia," by West Virginia State Society of Medical Technologists.
- "Comparative Studies of Photoelectric R.B.C. and Hb. with Hemocytometer Method," by Wisconsin Society of Medical Technologists.
- "Progress in Alabama," by Alabama State Society of Medical Technologists.
- "Oklahoma State Society."
- "Virginia State Society."

ANNOUNCEMENT

The Board of Directors of 1949-1950 announces the appointment of Miss Rose Mattheai to the position of full time Executive Secretary of the American Society of Medical Technologists and Editor-in-chief of the AMERICAN JOURNAL OF MEDICAL TECHNOLOGY. After September 1, 1949, all communications to the Executive Office, and to the journal, shall be sent to **2119 Arbor Ave., Houston 4, Texas.** Until that time, the Executive Office in Lafayette, Louisiana, will continue to function. The Board wishes to express its appreciation to Miss Tate and to Mrs. Willett for the work they have done during the past 5 years.

NEWS FROM STATE SOCIETIES

VIRGINIA

The annual meeting of the Virginia Society of Medical Technologists was held in the Medical School of the University of Virginia at Charlottesville, Virginia on May 7th, 1949. Dr. Tiffany of the United States Public Health Bacteriological Department spoke on "Recent Advances in Bacteriology" and Colonel Virgil Cornell of Walter Reed Hospital spoke on "WHAT is a Medical Technologist?" A business session was held after the program. Following a dinner at the Farmington Country Club a film on Bikini was presented to the group by Dr. John Yoe.

MASSACHUSETTS

A conference of the Massachusetts Association of Medical Technologists, Inc. met in Worcester on April 23. Although the Worcester District Medical Technologists Association, formed in 1943, became incorporated under the name Massachusetts Association of Medical Technologists and Laboratory Technicians on November 6, 1945, this was the first time that representatives from all parts of the state had come together. The newly formed associate groups centered in Boston, Fall River, Salem, Springfield and Pittsfield were well represented.

All registered M.T.s (A.S.C.P.) were invited to hear Mrs. Beatrice Allison, President of the New York Society of Medical Technologists, speak on the American Society of Medical Technologists. Mrs. Allison clearly differentiated between it and the Registry and told what the Society has done and hopes to do for us.

In the afternoon, Victor C. Vaughan, III, MD assistant to Louis K. Diamond, MD, spoke on "The Newer Serological Techniques for Iso-Antibodies" and Donald A. Nickerson, MD, the representative for the American Society of Clinical Pathologists in this region discussed the "Registry—Its Standards and How To Meet Them." Over 150 were present at the meetings and forty members signed the roll.

STATE SOCIETIES

ALABAMA: President: Drusilla Mullane, A.P.I., Auburn.
Vice President: Mary Ward, 2904 South 18th St., Birmingham.
Secretary: Mrs. Flora M. Herring, 930 So. 20th St., Birmingham.
Treasurer: Sara Douglas, 212 Mecca Ave., Birmingham.

ARIZONA: No organization.

ARKANSAS: President: Doris Thompson, University Hospital, Little Rock.
President-elect: Betty Rice, P.O. Box 2731, Little Rock.
Vice President: Sara Munn, 1224 Barber, Little Rock.
Secretary: Sister M. James (Poiriot), St. Bernard's Hospital, Jonesboro.
Treasurer: Mrs. Naomi Meek, 719 North 34th, Fort Smith.

CALIFORNIA: President: Barbara Isbell, Vet. Admin. Reg. Office, San Diego 1.
President-elect: Hazel Current, 918—17th St., Santa Monica.
Secretary: Alice Daniel, State Hospital, Modesto.
Treasurer: Ellen M. Bahr, Birmingham Veterans' Hospital Lab., Van Nuys.

COLORADO: President: Mrs. Virginia Wier, 525 Jackson St., Denver.
Secretary: Marguerite B. Pitinga, Memorial Hospital, Colorado Springs.
Treasurer: Rose Hackman, 4200 East 9th Ave., Denver.

CONNECTICUT: President: Anita Charbonneau, St. Joseph's Hospital, Stamford.
Secretary: Bertha Diem, 74 Douglas St., Hartford.
Treasurer: Alice Anderson, Middlesex Hospital, Middletown.

DELAWARE: President: Georgene M. Withers, 708 W. 20th St., Wilmington.
President-elect: Mr. Evelyn G. Scott, 4 Chaplain Ave., Wilmington 131.
Secretary-treasurer: Mrs. Helen Rairigh, 1693 Concord Pike, Wilmington 284.

Board of Directors: Mr. George A. Neville, Biochemical Research Foundation, Newark; Ruth M. Church, c/o Nurses' Home, Wilmington Gen. Hosp., Wilmington.

DISTRICT OF COLUMBIA: President: Mary Sproul, 9320 Jones Mill Road, Chevy Chase, Md.
President-elect: Mary Frances Gridley, 1801 N. Quinn St. No. 102, Arlington, Va.
Recording Secretary: Patricia Albright, 202 East Luray Ave., Alexandria, Va.
Corresponding Secretary: Mary Ann Marx, 7801 - 14th St., N.W., Washington, D.C.
Treasurer: Mrs. Martha F. Spiegel, 4810 S. 29th St., Arlington, Va.

FLORIDA: President: Eleanor Brenny, 384 Brent Bldg., Pensacola.
Vice President: Marie Colburn, 327 N.E. 18th St., Miami.
Secretary: Lois E. Hensley c/o Nurses' Home, Morrell Memorial Hospital, Lakeland.
Treasurer: Virginia Morgan, Box 696, Gainesville.

GEORGIA: President: Sadie Cartwright, 606 East 51st St., Savannah.
Vice President: Estelle Gee, St. Joseph's Infirmary, Atlanta.
Secretary: Mrs. Sarah Anderson, B2 Laboratory, University Hospital, Augusta.
Treasurer: Carolyn Martin, No. 3 Forrest St., Rome.

IDAHO: President: Virginia Woodhead, 1138 Bannock St., Boise.
 Vice President: Ruth Anglemire, 116 Seventh Ave., East, Twin Falls.
 Secretary: Florence Meili, 127 East Second Ave., Twin Falls.
 Treasurer: Mr. Foster W. Burke, 151 Second Ave., East, Twin Falls.

ILLINOIS: President: Ellen Skirmont, 5493 Cornell, Chicago 15.
 President-elect: Beth Armsey, 5208 Main St., Lombard.
 Recording Secretary: Ann Byrne, 1430 N. Dearborn, Chicago.
 Treasurer: Marie McCoy, 2270 West 69th St., Chicago.
 Executive Secretary: Eleanor Strack, 102 E. Chestnut St., Chicago.

INDIANA: President: Gladys Bainaka, 1158 North Holmes St., Indianapolis.
 Vice President: Ruth A. Morgan, 1859 N. Talbot, Indianapolis.
 Secretary: Constance Padden, 3630 N. Meridian St., Indianapolis.
 Treasurer: Mr. Willis M. Overton, 1334 Ringgold, Indianapolis.

IOWA: President: Inga Overland, 650-16th St., Des Moines.
 Vice President: Rachel Hall, St. Joseph's Mercy Hospital, Fort Dodge.
 Secretary: Eleanor Amberg, Broadlawns Gen. Hospital, Des Moines.
 Treasurer: Mrs. Mae Chader, Slater.

KANSAS: Mrs. Marjorie Kaufman, 230 No. Glenn, Wichita.
 President-elect: Eileen Ebel, 641 So. Kansas Ave., Wichita.
 Secretary: Mildred King, 1645 Park Place, Wichita 4.
 Treasurer: Margaret June Latimer, McPherson Co. Hosp., McPherson.
 Board of Directors: Dr. M. Anthony, Mercy Hospital, Parsons; Ruby Dounum, 408 So. Water, Wichita; Delores Maus, 321 Castor, Salina; Mrs. Allie Wilson, 222 East 9th, Horton; Ada Gregory, 504 So. Denver, El Dorado.

KENTUCKY: President: Mary Benedict Clark, 301 McCready Ave., Louisville 6.
 Vice President: Mr. Wade M. Marsh, Jr., 312 Hilltop, Lexington.
 Secretary: Mary Catherine Sullivan, 711 Hazel St., Louisville 3.
 Treasurer: Thelma L. MacIntyre, 221 Cotter Ave., Somerset.

LOUISIANA: President: Hermine Tate, Charity Hospital, Lafayette.
 Secretary: Carolyn E. Steir, 84½ Roosevelt Place, New Orleans 19.
 Treasurer: Mrs. Dorothy C. Preddy, St. Frances Sanitarium, Monroe.

MAINE: No organization.

MARYLAND: President: Mrs. Kathryn F. Dean, 835 Glenwood Ave., Baltimore 12.
 Vice President: Mrs. Rita F. Berry, 16 E. Mt. Vernon Place, Baltimore 2.
 Corresponding Secretary: Genevieve M. Clement, 911 N. Broadway, Baltimore.
 Recording Secretary: Willa Murphy, 2615 Edmondson Ave., Baltimore 23.
 Treasurer: Anne Hellen, 1211 Bolton St., Baltimore 17.

MASSACHUSETTS: President: Mrs. Elinor Judd, 452 Park Hill Drive, Boston.
 Vice President: Mrs. Reita N. Gormally, 761 State St., Springfield.
 Secretary: Marjorie Inman, 7 Oaks St., Worcester.
 Treasurer: Mr. Louis De Laura, Truesdale Hospital, Fall River.
 Board of Directors: Grace Foley, 13 Jenks St.; Marion Alcott, 64 Lincoln St., Watertown; Muriel Lee Gilkey, 55 Foster St., New Bedford.

MICHIGAN: President: Hazel L. Stoerck, 340 E. Grand Ave., Apt. 214, Detroit 7.
 Secretary: Laura J. Peterson, 123 So. Arlington, Kalamazoo.
 Treasurer: Josephine Russ, 423 Todd Ave., Reed City.

MINNESOTA: President: Mr. Aubrey Lewis, Mankato Clinic, Mankato. President-elect: Elizabeth Maclay, 1141—9½ Ave., S.E., Rochester. Secretary: Sister M. Emerita (Ohmann), San Gabriel's Hospital, Little Falls.

Treasurer: Arlene Magnussen, 806 Second St., S.W., Rochester. Board of Directors: Mrs. Martha Strolberg, Howard Lake.

MISSISSIPPI: President: Mr. Edward G. Michael, Houston Hospital, Houston.

President-elect: Mr. John N. Frazer, The Street Clinic, Vicksburg.

Secretary: Mrs. Betty L. Cain, The Street Clinic, Vicksburg.

Treasurer: Gladys Elmore, Mississippi State Board of Health, Jackson.

MISSOURI: President: Frances Moore, P.O. Box 615, St. Joseph 10. Vice President: Sister M. Leo Rita, 6420 Clayton Road, St. Louis.

Secretary: Jean Rutherford, 1837 Pendleton, Kansas City 1.

Treasurer: Patricia Thomas, 6148 Tennessee, St. Louis.

Board of Directors: Mildred Oswald, 4552 Lacleda, St. Louis; Anne J. Sommer, 1325 So. Grand, St. Louis; Emma Mae Baldwin, St. John's Hospital, Springfield.

MONTANA: President: Sister Barbara Clare (Hageman), St. Patrick's Hospital, Missoula.

Vice President: Eloise Patten, 734 So. 2nd St., Missoula.

Secretary-Treasurer: Grace McConnell, 602 East Kent, Missoula.

NEBRASKA: President: Mrs. Mary Gibb, 5019 Huntington Ave., Lincoln.

President-elect: Inez Roesky, 2241 Laramore Ave., Omaha.

Secretary: Mrs. Ida Blore, 1721 "F" St., Lincoln.

Treasurer: Virginia E. White, 3079 So. 33rd, Omaha.

NEVADA: No organization.

NEW HAMPSHIRE: President: Beverly Bates, Elliot Hospital, Manchester.

Vice President: Sister M. Aybert, St. Louis Hospital, Berlin.

Secretary: Annie Clark, Mary Hitchcock Memorial Hospital, Hanover.

Treasurer: Mr. Melvin Cooley, Franklin Hospital, Franklin.

NEW JERSEY: President: Mrs. Margaret B. Priestley, Englewood Hospital, Englewood.

Vice President: Florence Cook, Fitkin Memorial Hospital, Neptune.

Secretary: Louise Pallotta, St. Michael's Hospital, Newark.

Treasurer: Mrs. Elizabeth Kauderer, Municipal Hospital, Camden.

NEW MEXICO: President: Sister Joan of Arc (Allard), St. Anthony's Hospital, Las Vegas.

Secretary: Vivian Dunstan, Box 83, Taos.

Treasurer: Mrs. Lorraine Moody Harris, 1210 E. Tijeras Ave., Albuquerque.

Board of Directors: Mr. Charles Lewellan, Veterans' Hospital, Albuquerque.

NEW YORK: President: Anne Keenan, 31 Cuyler Ave., Albany.

Vice President: Ruth Rothschild, 832 Park Ave., Apt. 15, Syracuse.

Recording Secretary: Anne Anderson, 1230 Boston Road, Bronx 56, N. Y. C.

Treasurer: Rhoda Rachman, 59 Crestwood Ave., Buffalo 16.

Board of Directors: Sister Mary Clare (Heath), 415 W. 51st St., N. Y. C. 19; Mrs. Beatrice Allison, Niagara Sanitorium, Lock Port.

NORTH CAROLINA: President: Arline Steinacher, Memorial Hospital, Charlotte.

President-elect: Dorothy McGee, Rex Hospital, Raleigh.

Secretary: Sister M. Rosaria (Brennan), Mercy Hospital, Charlotte.

Treasurer: Mrs. Clara B. New, Veterans' Hospital, Fayetteville.

Board of Directors: Mittie Pickard, Med. Bldg., Chapel Hill.

NORTH DAKOTA: President: M. Claire Murray, Box 476, Jamestown. Secretary-Treasurer: Sister Moira (Paulus), St. Alexius Hospital, Bismarck.

OHIO: President: Mrs. Bertina B. Orsburn, Children's Hospital, Columbus. President-elect: Charlotte Thumm, 3323 Denison Ave., Cleveland 9. Secretary: Sister Jean Clare (Kenney), Good Samaritan Hospital, Cincinnati. Treasurer: Bessie Keating, 55 W. Hebble St., Dayton. Executive Secretary: Mrs. Ruth U. Clark, Grant Hospital, Columbus. Board of Directors: Sister Eugene Marie (Carpe), Good Samaritan Hosp., Cincinnati.

OKLAHOMA: President: Margaret Haraway, 427 Osler Bldg., Oklahoma City. President-elect: Marie J. Wilson, 1512 S. Florence Place, Tulsa. Secretary: Helen Jones, 1115 Medical Arts Bldg., Oklahoma City. Treasurer: Anne Adwan, 1440 N.W. 30th, Oklahoma City. Board of Directors: Sister M. Charlotte (Rohr), St. Mary's Hospital, Enid. Elizabeth Parks, 412 N.W. 12th St., Oklahoma City.

OREGON: President: Mrs. Elsa Thompson, 7609 S.W. 33rd Ave., Portland. President-elect: Mary Barbara Godfrey, 2045 N. Farragut, Portland. Secretary: Mary Susan Reik, 3545 N. Borthwick, Portland. Treasurer: Helen Janes, 4209 N.E. Laurelhurst, Place, Portland.

PENNSYLVANIA: President: Mr. Harry Langer, Canansburg General Hospital, Canansburg. Secretary: Elizabeth M. Heck, 958 North 5th St., Philadelphia 23. Treasurer: Kathryn Simmons, 806 Summit Avenue, Prospect Park.

SOUTH CAROLINA: No organization.

SOUTH DAKOTA: President: Sister M. Mauritia (Schuermann), Sacred Heart Hospital, Yankton. Vice President: Arthur R. Lundquist, Box 96, Webster. Secretary-Treasurer: Sophia A. Rados, State Sanitorium, Sanator.

RHODE ISLAND: No Organization.

TENNESSEE: President: Bernice Triplett, 1868 Vinton Avenue, Memphis. Vice President: Rebecca Stallworth, 12 So. Bellevue, Memphis. Secretary: Rosemary Legay, St. Joseph's Hospital, Memphis. Treasurer: Sue Nell Adkins, 118 - 21st Ave., So., Nashville.

TEXAS: President: Mrs. Phyllis Shaw, 123 Cromwell Drive San Antonio. President-elect: Dorothy Patras, Harris Memorial Methodist Hospital, Ft. Worth. 1st Vice President: Elsie Urbantke, 1700 Palma Plaza. 2nd Vice President: Elise Cox, Hendrick Memorial Hospital, Abilene. Secretary: Mrs. Neenah Lang, 1709 San Antonio, Office 3, Austin. Treasurer: Ruth Guy, Buchanan Blood & Plasma Center, Dallas. Board of Directors: Mrs. Olive Pohlen, 1529 North 5th St., Waco; Mrs. Florence Strang, Dow Hospital, Freeport; Jean Stubbins, John Sealy Hospital, Galveston; Betty McGrew, 2001-A Whitis, Austin; Faith Wayne, 3306 Carpenter, Dallas; Mildred Peel, Univ. of Houston, Bldg. 37, Apt. 11, Houston; Lucile Harris, Hendrick Memorial Hospital, Abilene.

UTAH: President: Katherine Dean, St. Benedict's Hospital, Odgen. President-elect: Adrie E. Langan, St. Benedict's Hospital, Odgen. Secretary: Marguerite George, Holy Cross Hospital, Salt Lake City. Treasurer: Mrs. Helen Evans, 1175 Lake St., Salt Lake City.

VERMONT: President: Ina Maxson, Univ. of Vermont, School of Medicine, Burlington.

Secretary: Mary E. Breen, 3 So. Willard St., Burlington.

Treasurer: Mrs. Phyllis Muerlin, School St., Essex Junction.

VIRGINIA: President: Eleanor Rawls, 800 Wainwright Bldg., Norfolk. Vice President: Mrs. Frances Crouch, 1405 Hillcrest Ave., Roanoke. Corresponding Secretary: Margaret Bryan, Riverside Hospital, Newport News.

Recording Secretary-Treasurer: Joy Austin, 10 Oakhurst Circle, Charlottesville.

Advisory Board: Harriet Howe, RFD #13, Box 29, Richmond; Claire Hutcher, 4004 Hermitage Road, Richmond; Eleanor McClain, Dept. of Public Health, Danville.

WASHINGTON: President: Neva Lyness Johns, 401 Security Bldg., Olympia.

Vice President: Mrs. Eugene S. Schneider, Tacoma General Hospital, Tacoma.

Secretary: Edna B. Wilcox, c/o Tenino Lumber Co., Tenino.

Treasurer: Marjorie Moss, 7711 Forest Drive, Seattle.

WEST VIRGINIA: President: Constance L. Peterkin, 907 Short Avenue, Fairmont.

Secretary: Margaret L. Wilson, 522 Main St., Charleston.

Treasurer: Mr. Gordon S. Starkey, Myers Clinic, Philippi.

WISCONSIN: President: Grace Ballard, 925 N. 13th St., Milwaukee.

President-elect: Betty Joseph, 949 N. 23rd St., Milwaukee.

Secretary: Margaret Foley, 2029 W. State St., Milwaukee 3.

Treasurer: Mrs. Margaret Brei, 8435 Kenyon Ave., Wauwatosa 13.

WYOMING: President: Patricia Ruth Gerlach, 1833 East "B" St., Torrington.

Secretary: Kathryn Eaton, Memorial Hospital, Cheyenne.

Treasurer: Jane B. Smyth, Cross U Bar Ranch, Big Horn.

Please send any notice of changes or corrections in list of officers to the Editorial and Executive Office of A.S.M.T., 6544 Fannin St., Houston, Texas, after September 1, 1949.

OPPORTUNITIES

Technician to work with research project involving bacteriology, serology and some parasitology; college of medicine, eastern university. MT 7-1.

Biochemist; young man with medical or Ph.D. degree; research appointment, university medical school; opportunity for further training. MT 7-2.

Tissue technician qualified to handle eye and brain work and, also, hematological technician; large teaching hospital; outstanding laboratory staff, university medical center; Middle West. MT 7-3.

Senior pharmacologist; Ph.D. or M.D. with pharmacological research experience; should be qualified to initiate and develop pharmacological research program, pharmaceutical company; East. MT 7-4.

Well trained and experienced X-ray and laboratory technician; office of general practitioner; town of 10,000; winter resort area, Southwest; starting salary around \$300. MT 7-5.

Young woman, university graduate, who can be trained in tissue culture work; chemistry training required; advantageous if qualified in cytology or histology; university medical center; East. MT 7-6.

Hematology technician to assist hematologist with bone marrow aspirations and, also, biochemist; laboratories of university medical school; Middle West. MT 7-7.

Registered laboratory technician; advantageous if trained in special cancer test (Papponicoff); small hospital, busy laboratory department; Hawaii. MT 7-8.

(In requesting information concerning these appointments, we shall appreciate your mentioning the key numbers)

M. BURNEICE LARSON

32nd Floor, Palmolive Building

Director, The Medical Bureau

Chicago, Illinois

SELECTED OPPORTUNITIES

(In requesting information please refer to key numbers)

Bacteriologists: (a) City health department, convenient midwest location; \$3600 yearly. Male. Degree. (b) State health department, southeast; five days, 35½ hour week. Ph.D. required, \$5000 yearly.

Combination Laboratory & X-ray Technicians: (a) well established physician northwestern university town; \$400 monthly. (b) Fifty bed approved hospital, pleasant college town; midwest, \$350. (c) Forty bed approved hospital, southwest, \$350. (d) Medical Department large Texas oil company; \$300 increases. (e) Doctor's office, pleasant mountain resort northern California; hours 9 to 5; 5½ day week, \$300 monthly. (f) Seventy bed approved hospital eastern university town; attractive salary.

Medical Technologist: (a) Six-man diagnostic clinic eastern capital; \$3000 yearly. (b) California hospital-clinic not far from state capital; forty hour week, \$3300 yearly plus extras. (c) Well established clinic, southern California; 5½ day week; opportunity for advancement; \$4000 yearly. (d) Twenty-five man clinic northwestern state capital; modern equipment, \$3600 yearly

up. (e) 200-bed approved general hospital, Midwest, \$3300 yearly. (f) 150 bed general hospital near Cincinnati; well qualified in bacteriology or blood chemistry; \$3600 to \$4000. (g) Office of specialist, large midwest city; \$3600 up. (h) Registered; 90 bed approved hospital attractive southwestern location; \$3600 yearly. (i) Night Technician; hours 6 to 12 p.m., approved hospital midwest college town. \$300 per month.

Head Technicians: (a) Clinical Laboratory, five-man group, private hospital; 5½ day week, no calls, California. \$400 monthly. (b) 200-bed approved Florida hospital near Gulf of Mexico; \$4200 yearly. (c) 500-bed general hospital with certified pathologist; \$3600 up. (d) 100-bed general hospital, East Coast; minimum \$3600 yearly. (e) 200-bed Michigan hospital; pleasant college town adjacent state capital; \$3600 to start. (f) Male technician; 100-bed Pennsylvania hospital, \$3600. (g) Degree, registered, able to teach approved student training school; 300 bed eastern hospital, \$4200 to start. (h) New mental hospital adjacent Honolulu, Hawaii; \$2,750 maintenance.

(We have many other excellent opportunities—please write for complete list—strictly confidential)

WOODWARD MEDICAL PERSONNEL BUREAU

ESTABLISHED 1896

ANN WOODWARD, Director

185 N. Wabash Ave.

Chicago 1, Ill.

Please Mention Publication When Writing Advertisers

to
to
s-
ty

so,
al-
tal
al-

ng
wn
in-
-

ted
ted
th.

of
of
lia-

ory
one
ent

and
ped
iddle

try
I in
ency:

the

lads

Mid-
capital
ology
Office
up.
al at-
early.
. ap-
9 per

five-
ok, no
d ap-
mexico;
with
so-bed
\$2000
ement
500 to
nmay-
stered,
chool;
t. (h)
i, Ha-

ential)

1, III.